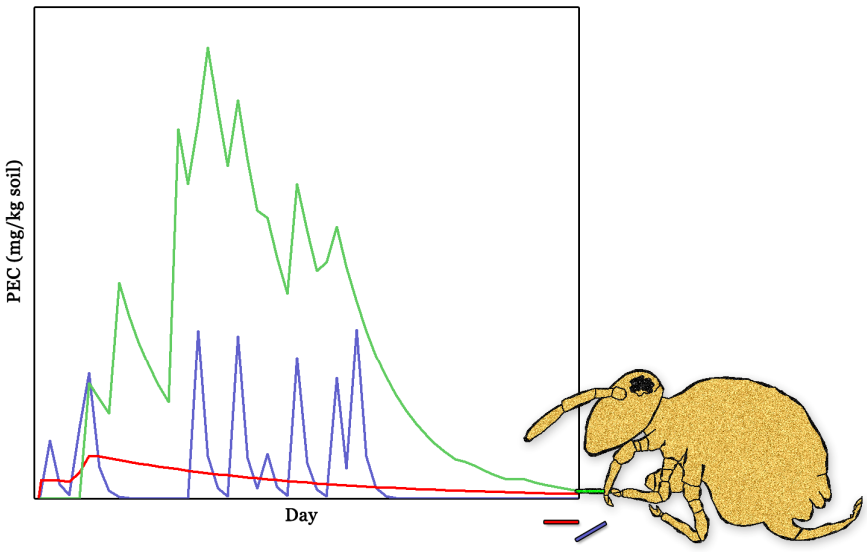
Ecotoxicological effects on structure and function of aquatic and terrestrial ecosystems



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DOTTORATO DI RICERCA IN SCIENZE AMBIENTALI

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Ecotoxicological effects on structure and function of aquatic and terrestrial ecosystems

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"Nature is not only more complex than we think.
It is more complex than we can think"

Egler FE, *The Way of Science. A philosophy of Ecology for the Layman*, 1970

Contents

CHAPTER I

General introduction

1.1. Traditional risk assessment procedures

Ecotoxicological risk assessment can be defined as the quantification of the probability that adverse effects on ecosystems occur or are expected after exposure to a contaminant (OECD, 1989). In current regulations simplified procedures for performing risk assessment have been developed. To comply the requirements of European legislation, official procedures are reported in the Technical Guidance Document (TGD) in support of Commission Directive 93/67/EEC, Commission Regulation (EC) No. 1488/94 and Directive 98/8/EC of the European Parliament and of the Council (EC, 2003). Risk assessment procedures for Plant Protection Products (PPP) are reported in the Annex IV of the Directive 91/414/EC (EC, 1991). These procedures comprise three steps: exposure assessment, characterisation of the effects and risk characterisation.

The first step is characterisation of the exposure in the environment, which can be assessed through two methods: direct measurements and model application. The result of both would be a Predicted Environmental Concentration (PEC) in an environmental compartment.
The experimental approach provides precise concentrations of a chemical present in the different environmental matrices. Therefore, ideally, it is the best approach for having results that actually reflect the environmental situation. However, some shortcomings have to be highlighted. Firstly, it is an *a posteriori* information, because the matrix to be analysed is sampled after the contamination occurred, thus it does not allow any prevention. Moreover, at large scale it is pretty impossible to use the approach, both for too large monitoring campaigns and for problems in upscaling the point information. Finally, it is limited to a given time, so, to have a record of the time variability, the monitoring should be repeated with a frequency that is a function of the disappearance of the chemical in the compartment (degradation, volatility, etc.).
On the contrary, predictive models allow the prediction of distribution and fate pattern of chemicals, and are an *a priori* method of PEC estimation. They need a lot of input data referred to the properties and the use patterns of the chemicals involved in the assessment, the environmental characteristics of the investigated compartment and other environmental parameters (e.g. meteorological information). The construction of defined scenarios is thus taken into account. The FOCUS (FOrum for the Co-ordination of pesticide

fate model and their Use) working group drew standardised scenarios for risk assessment in surface and groundwater to be used in exposure assessment representative of the different environmental and climatic European conditions (FOCUS, 2001; 2002). One of the major advantages is that predictive models allow working at different scales: local, regional or even continental. Anyway, the results are a prediction and not a precise measurement, thus models need to be accurately calibrated and validated through environmental measurements.
Both the methods possess advantages and disadvantages, thus the best strategy would be integrating them, using models to plan monitoring and understand data, and using measurements for checking results.

The second step is the characterisation of the effects that a chemical can cause. Firstly, the subjects of the procedure have to be identified. Risk assessment refers to organisms and for each compartment indicator species were listed, taken as representative of all trophic levels. For example for the freshwater compartment algae has been identified as representative of primary producer, *Daphnia* of primary consumer and fish of secondary consumer. In the other compartments indicator organisms may be:

- microorganisms in sewage treatment plants,
- dwelling organisms in sediments,
- earthworms and seldom springtails or mites in soil,
- bees, birds and mammals for terrestrial ecosystems.

The effect on indicator species are expressed as ecotoxicological endpoints, as e.g. EC_{50} (Effect Concentration on 50% of the population) or NOEC (No Observed Effect Concentration). A possibility to extrapolate the results to the entire community is the calculation of a PNEC (Predicted No Effect Concentration) by applying safety factors to ecotoxicological endpoints.
Also for effects, beside the direct measurements for a few substances, predictive models can be used. QSARs (Quantitative Structure – Activity Relationships) are tools used for a variety of purposes, among which the prediction of ecotoxicological endpoints. These models are correlation equations between an ecotoxicological endpoint experimentally determined for a series of chemicals and some molecular characteristics (physical-chemical properties, structure, etc.). The equations allow predicting the endpoint for other chemicals of comparable structure.

For risk characterisation, the third step, both the exposure and effect data are compared. According to Directive 91/414/EC, risk is quantified through the calculation of the TER (Toxicity/Exposure Ratio), i.e. the ratio between an exposure indicator (e.g. PEC) and an indicator of the effect (e.g. EC_{50}). In the case of organisms for which exposure is difficult to quantify (e.g. pollinators) risk is calculated as HQ (Hazard Quotient), the ratio between

pesticide application rate and LD_{50} for bees, without any assessment of actual exposure.
The TGD (EC, 2003) proposes a tiered approach for risk assessment: firstly a PEC/PNEC ratio is calculated. Depending on the result, the procedure ends with an indication of no risk or of further analyses to be performed, in order to:

- determine whether additional information would lead to a refine of ratios,
- ask for additional tests,
- or refine the ratio.

In order to compare and rank different compounds, the use of risk indexes is a powerful tool. Indexes for PPPs were proposed by Finizio *et al.* (2001). They combine the risk assessment performed on the single component of an environmental compartment into a unique value through the application of suitable algorithms.

The procedures described are powerful tools to assess the potential risk for an ecosystem and to rank chemicals according to the threat that they pose. They are schematic, simplified and standardised methods, because they have to be included, and actually they are, in regulations.
Anyway, two main shortcomings have to be highlighted.
The risk assessment methods described above refer to a "general" environmental scenario, they are not site-specific. Indeed, the objectives of the regulations are to give indications suitable to be used in the whole European territory. For site-specific risk assessment, all the steps described above have to be refined on the actual situation that has to be evaluated. Thus the right scale has to be assessed, the emission patterns have to be characterised both in space and time to provide the right input data for model applications, environmental parameters have to be carefully assessed and described in order to evaluate their influence on the variability of the predicted exposure. The spatial variability of the parameters can be assessed using GIS (Geographic Information Systems), that allows also to map monitoring data and PECs and to adapt predictive approaches to environmental conditions (e.g. Verro *et al.*, 2002). Also the Water Framework Directive (WFD; EC, 2000) proposes the use of site-specific methods as a tool to assess the quality of the water bodies, based on the structure and functioning of biological communities. The WFD thus highlights the effects on the community rather than the causes, changing the regulatory perspective. Thus also the targets should be refined and the characterisation of the exposed biological community is needed. Also the ecosystem characterisation can be taken into account in GIS-based models, as shown in Sala and Vighi (2008).
The problem of the targets is the second main shortcoming of the risk assessment procedures as proposed by European regulations. The methods

focus on a few indicator species, upscaling to the community level by applying safety factors. The procedure is relatively simple and practically applicable, thus it has an undoubted pragmatic value for regulation purposes. However the capacity to predict the actual consequences on natural communities is poor (Van Straalen, 2003). Indeed, interactions among populations within the community or indirect effects, both positive and negative, of a contaminations are not taken into account. Moreover, the laboratory studies needed for assessing effects, are carried out in controlled conditions, for repeatability and reproducibility purposes, while in the real environment parameters fluctuations may act as additional stressors.

1.2. The need for more "ecological realism"

Considering the shortcomings of the traditional risk assessment procedures, ecotoxicology should move towards an higher ecological realism, as highlighted by several authors (Calow, 1998; Chapman, 2002, Van Straalen, 2003; Vighi *et al.*, 2006; Van den Brink, 2008).
Firstly, the focus should be put not only on species as targets, but also the structure and functioning of an ecosystem has to be taken into account. For example, a small changing in the structure of an ecosystems, such as the depletion of a taxon, could lead to great changes in the functions. It is the case of the species carrying out ecosystem services, as e.g. pollinators. Reversely, small changes in function could be linked to great variation in the structure, for the redundancy of the species responsible of a particular ecological process. The WFD is, again, an example of this changed perspective. The directive aims to protect water bodies not for their use as resource by humans, but for their intrinsic value, guaranteeing their structure and functioning.
Another recent approach in the direction of increasing the ecological realism in ecotoxicology is the shift from the concept of sensitivity to that of vulnerability (De Lange *et al.*, 2009; 2010), that is the degree to which a system is susceptible to, and unable to cope with, injury, damage or harm. The concept could refer to different hierarchical levels, from organisms, to population, community, ecosystem and landscape. Williams and Kapustka (2000) defined vulnerability of an ecosystem as its potential to modulate responses to stressors in time and space, that is determined by the characteristics of the ecosystem that comprise different levels of organization. For assessing the vulnerability of an ecosystem individual organisms together with structural and functional relations among them and with the abiotic environment are involved. De Lange *et al.* (2009) focused on species vulnerability, being populations the first level ecologically relevant that react to a perturbation. It is defined as the extent to which species are affected by field effects of a stressor as result of their ecological

characteristics related to toxicological sensitivity, potential exposure to the stressor and recovery capability. Thus, according to Van Straalen (1994), vulnerability can be defined as function of three components: sensitivity, susceptibility to exposure and recovery potential.
A tool for predicting vulnerability is, thus, the use of biological traits. They are morphological, life cycle, ecological, physiological attributes of an organism that describe their physical characteristics, ecological niche and functional role within the ecosystem (Baird and Van den Brink, 2007; Baird *et al.*, 2008). The hypothesis is that sensitivity to a stressor may be predicted by those traits. The concept arise from the necessity to predict the effect of a stressor for all the species comprised in an ecosystem, not only those for which ecotoxicological tests are carried out. This would lead to make a step forward from the use of a few indicator species, need that was highlighted in section 1.1. The major advantages of using this tool are to overcome a taxonomy based risk assessment, by focusing on the characteristics making an organism vulnerable to a stressor and on the link between prediction on ecological function and mechanisms of action of different stressors (Baird *et al.*, 2008).

1.3. Structure of the work

The present work starts from traditional risk assessment procedures for aquatic and terrestrial ecosystems and aims to put more "ecological realism" into them, also with studies on natural communities. The new tools presented in section 1.2, vulnerability analysis and trait-based approach, have been applied.
Three environmental compartment are taken into account: freshwater, terrestrial aboveground and terrestrial belowground.

- A risk assessment for the aquatic compartment is presented in chapter II, and the main critical points are highlighted. The results are compared with two studies on communities: one laboratory study with environmental matrices and one previous work by Bonzini *et al.* (2008) investigating natural communities in field.
- Soil risk assessment has been performed (chapter III), highlighting the major critical points regarding the effect assessment and the organisms taken into account.
- In chapter IV exposure assessment for pollinators is described, according to Barmaz (2009) and Barmaz *et al.* (2010), and validated. Main differences among the three compartments are also reported.
- In chapter V a microarthropod community exposed to agrochemical applications is investigated and the main trends of the different taxa described according to stressors. Also indirect effects are taken into account.

- In chapter VI an attempt to identify the consequences of the stressors on the food web and separate indirect from direct effect is made.
- The new tools in ecotoxicology, trait based approach and vulnerability analysis, are applied on the microarthropod community, in chapters VII and VIII respectively. The results are compared, also with the trends described in chapter V.

References

Baird DJ and Van den Brink PJ, 2007. Using biological traits to predict species sensitivity to toxic substances. *Ecotox Environ Saf* 67: 296-301.
Baird DJ, Rubach MN, Van den Brink PJ, 2008. Trait-based ecological risk assessment (TERA): the new frontier? *Integr Environ Assess Manag* 4: 2-3.
Barmaz S, 2009. Plant protection product risk assessment: distribution and experimental validation in terrestrial ecosystems. PhD thesis, Doctorate in Environmental Sciences (XXII cycle), Faculty of Mathematical, Physical and Natural Sciences, University of Milano – Bicocca, Milano, Italy, 113 pp. http://hdl.handle.net/10281/7503.
Barmaz S, Potts S, Vighi M, 2010. A novel method for assessing risks to pollinators from plant protection products using honeybees as a model species. *Ecotoxicology* 19: 1347-1359.
Bonzini S, Finizio A, Berra E, Forcella M, Parenti P, Vighi M, 2008. Effect of river pollution on the colonisation of artificial substrates by macrozoobenthos. *Aquat Toxicol* 89: 1-10.
Calow P, 1998. Ecological risk assessment: risk for what? How do we decide? *Ecotox Environ Saf* 40: 15-18.
Chapman PM, 2002. Integrating toxicology and ecology: putting the "eco" into ecotoxicology. *Mar Pollut Bull* 44:7-15.
De Lange HJ, Lahr J, Van der Pol JJC, Wessels Y, Faber JH, 2009. Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. *Environ Toxicol Chem* 28: 2233-2240.
De Lange HJ, Sala S, Vighi M, Faber JH, 2010. Ecological vulnerability in risk assessment – a review and perspectives. *Sci Total Environ* 408: 3871-3879.
EC (European Commission), 1991. Council Directive 91/414/EEC concerning the placing of plant protection products on the market. *Off J Eur Communities* L 1991; 230 19/8/1991.
EC (European Commission), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in field of water policy. Official Journal of the European Communities, L 327, 22/12/2000.

EC (European Commission), 2003. *Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC for the new notified substances and the Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC on biocides* second ed. European Commission, Luxembourg, Luxembourg, Part 1, 2 and 3, 760 pp.

Finizio A, Calliera M, Vighi M, 2001. Rating systems for pesticide risk classification on different ecosystems. *Ecotox Environ Saf* 49: 262-274.

FOCUS, 2001. *FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC*. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.

FOCUS, 2002. *FOCUS groundwater scenarios in the EU review of active substances*, Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000 rev.2, 202pp.

OECD (Organisation for Economic Co-operation and Development), 1989. *Report of the OECD workshop on ecological effect assessment*. OECD, Paris, France, 67 pp.

Sala S and Vighi M, 2008. GIS-based procedure for site-specific risk assessment of pesticides for aquatic ecosystems. *Exotox Environ Saf* 69:1-12.

Van den Brink PJ, 2008. Ecological risk assessment: from book-keeping to chemical stress ecology. *Environ Sci Technol* 42:8999-9004.

Van Straalen NM, 1994. Biodiversity in ecotoxicological responses in animals. *Neth J Zool* 44:112-129.

Van Straalen NM, 2003. Ecotoxicology becomes stress ecology. *Environ Sci Technol* 37: 325-330A.

Verro R, Calliera M, Maffioli G, Auteri D, Sala S, Finizio A, Vighi M, 2002. GIS-based system for surface water risk assessment of agricultural chemicals. 1. methodological approach. *Environ Sci Technol* 36:1532-1538.

Vighi M, Finizio A, Villa S, 2006. The evolution of the environmental quality concept: from the US EPA Red Book to the European Water Framework Directive. *Environ Sci Pollut R* 13: 9-14.

Williams LRR, Kapustka LA, 2000. Ecosystem vulnerability: a complex interface with technical components. *Environ Toxicol Chem* 19: 1055-1058.

CHAPTER II

Site specific risk assessment for individual chemicals and mixture in an agricultural river basin and intersection with ecotoxicological tests on environmental samples

Abstract

The importance of site-specific risk assessment for surface waters has been recognised. Within the European project NO MIRACLE this tool has been applied to a master case area. A risk assessment procedure for surface waters has been developed and applied on a small river basin, on the basis of previous works done in the same area. The risk given by individual chemicals was predicted both for drift and for runoff processes, also applying an aggregated risk index. The mixture composed by the plant protection products and its time course were characterised. The contamination follows a pulse pattern, with some high peaks, but the 80% of the mixture is always composed by a few chemicals. Some ecotoxicological tests were conducted by partners of the NO MIRACLE project and the results were compared with the theoretical risk assessment done. For fishes it seems that the results obtained by the partners follows the theoretical risk prediction. A comparison with a previous study on natural macrozoobenthos communities by Bonzini *et al.* (2008) has been done.

Keywords: site-specific, freshwater, risk assessment, ecotoxicological tests

2.1. Introduction

Pollution of water bodies is a problem that has been widely treated in ecotoxicology, since 1970s, when the discipline was born. It has been passed from the effects given by the release of chemicals in the environment at the sub-acute or sometimes even acute level, to the regulation of the majority of the substances and consequently a great reduction of these high effects and the need of more refined approaches. Also the aim of protection of the water bodies changed, from Water Quality Criteria (WQC, US EPA, 1974), developed to allow major uses of the water resource, like drinking or fishing, and focusing on the control of the agent (chemical or physical), to the Water Framework Directive (WFD, EC, 2000) that overcomes the previous concept and focuses on the effects on the ecosystem (Vighi *et al.*, 2006). WFD changed the prospective to the problem, also changing the aim of protection: water bodies should be protected not as resources that are used by men, but as environmental goods themselves, structure and function of whom should be guarded.
A group of chemical that can pose a risk for freshwater, due to its intentional release in the environment is the class of plant protection products (PPP). They are produced with the aim of being toxic for a certain adverse organism, and of being used in the open fields, on crops. Their application patterns and their properties can make them able to move to other compartments, including water bodies. Thus, they can pose a risk to the freshwater environment. Moreover, normally plant protection products are used in combination, on the same crop, resulting in complex mixtures of pesticides released in the environment.
In the WFD site-specific procedures are highlighted as a tool to characterise the ecological status of water bodies. Also the relatively new regulation on chemicals REACH (Registration, Evaluation and Authorisation of CHemicals, EC, 2006) recognise the need of site-specific risk assessment. In site-specific situation, GIS (Geographic Information Systems) could be a useful tool to manage the complexity of environmental data (see e.g. the prediction of chemical distribution and fate in the GREAT-ER procedure, Feijtel *et al.*, 1997; Schowanek *et al.*, 2004). A GIS-based procedure for mapping the risk in surface waters by plant protection products used in agriculture at the local scale was developed by Verro *et al.* (2002), updated by Sala and Vighi (2008), validated by Bonzini *et al.* (2006) and applied by Verro *et al.* (2009a, b) in a river basin with a high agricultural pressure.

The activity presented here was done within the NO MIRACLE (Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe) project of the European Commission. In the productive season 2008 a unique site was identified as a master case for activities on the aquatic and terrestrial environments (see chapter III and from V to VIII), in the same basin used by

Bonzini *et al.* (2006; 2008) and Verro *et al.* (2009a, b). In the master case a theoretical risk assessment has been done, to be intersected with activities on biological communities (Langer-Jaesrich and Scheil, personal communication; chapter V), for the observation in field of the real effects on biological communities given by the use of plant protection products.

2.2. Materials and methods

2.2.1. Site description

River Meolo is a small resurgence river, 17 km in length, located in Veneto region (North-East Italy) and is one of the drainage basins supplying the Venice lagoon. It flows in a flatland, with altitudes ranging between 3 and 31 m a.s.l.. Being a groundwater fed river, its average water flow is relatively constant, about 3.1 m^3/s (Verro *et al.*, 2009a), with relatively small variability due to precipitations. Its catchment covers an area of 2878 ha, which is 95% cultivated.
In the basin, the same site was chosen for the theoretical risk assessment in water and in soil (see chapter III). The sampling point is located nearly at the half of the river course, directly downstream the vineyard used for the soil activities (see chapters III and from V to VIII). Figure 2.1 shows the river basin, the sub-basin closing point (used also as a sampling point for water and sediments) and an additional sampling station in the upper part of the basin. The sub-basin created (upper Meolo basin) is 5/11 of the river Meolo catchment and covers 1308 ha, 70% cultivated. The major crops are maize (27%), soybean (8%), vineyard (20%) and wheat (15%), from which derives a high pesticide usage and contamination in the river. Risk assessment was performed for river Meolo in the sub-basin outlet.
Meteorological data were collected from meteorological station located 1.5 km from the field station (for precipitations see table 2.1).
Another sampling station has been identified as reference, in the upper part of river Livenza, a river as similar as possible to Meolo, that flows in Friuli Venezia Giulia region, North-East Italy. Livenza is a resurgence river as Meolo, flowing in the same geographic area and, although the river course is longer and bigger than Meolo, in the upper part it exhibits similar morphological and physical characteristics. The majority of its basin is on hills, but it can be considered a flatland river, being its spring situated at 25 m a.s.l., and the altitude of the studied area ranging from 25 to 15 m a.s.l. (Bonzini *et al.*, 2008). In Upper Livenza basin very few agricultural practices are present, it is located in an environmentally protected area and thus the pesticide contamination is negligible. In 2004 only a few hectares were cultivated: 6.7 ha for maize, 7.8 ha for grapevine and 0.5 ha for potato, covering respectively 0.5%, 0.6% and 0.04% of the catchment (Bonzini *et*

al., 2008). On this river samples were taken, but risk due to pesticides was not assessed, due to the negligible load.

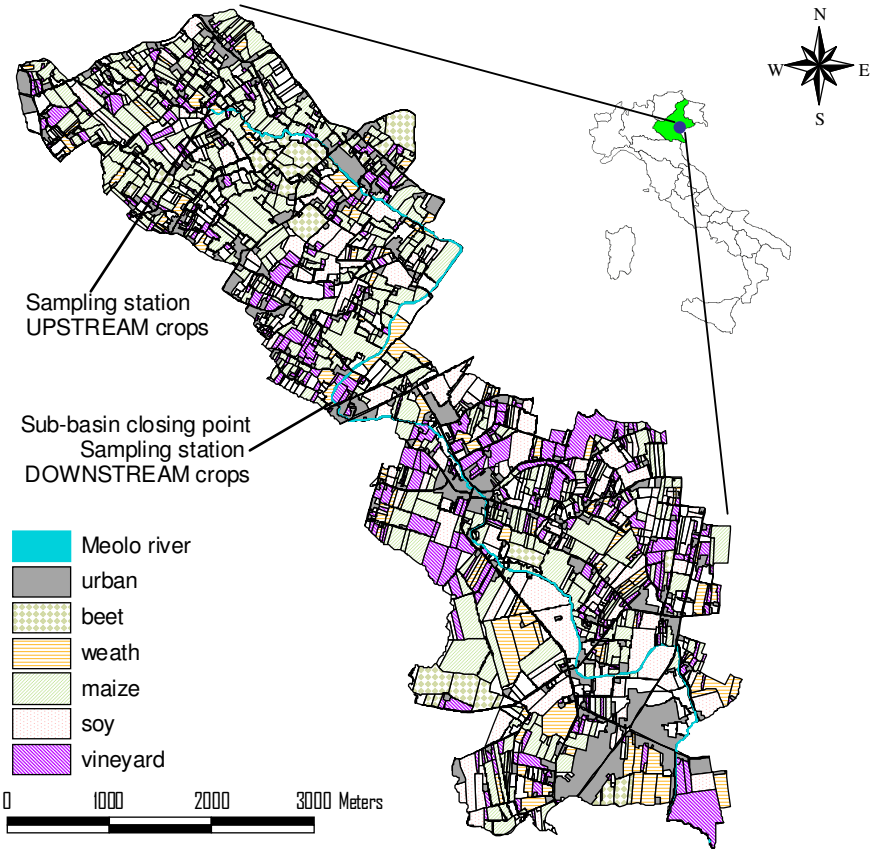


Figure 2.1. Meolo river basin (with 2004 land cover, data from Bonzini *et al.*, 2006) and stations used for the activity.

Table 2.1. Rainfall measured from April to September 2008 by the meteorological station located in San Biagio di Callalta (TV).

Date	Rain (mm)	Date	Rain (mm)	Date	Rain (mm)
5-Apr	0.2	18-May	49.2	14-Jul	17.8
6-Apr	0.8	19-May	0.4	17-Jul	0.4
9-Apr	0.2	20-May	1.2	18-Jul	2.4
10-Apr	2.8	23-May	12.2	22-Jul	0.8
11-Apr	6	25-May	1	28-Jul	2.2
12-Apr	3.8	4-Jun	16	8-Aug	13.4
13-Apr	0.2	5-Jun	26.8	15-Aug	2.4
15-Apr	1.2	6-Jun	0.8	16-Aug	13.6
16-Apr	0.8	7-Jun	0.6	23-Aug	6.8
18-Apr	6.8	11-Jun	7	24-Aug	0.4
19-Apr	4.6	12-Jun	0.4	2-Sep	1.2
21-Apr	11.4	14-Jun	4	4-Sep	12.2
22-Apr	0.4	17-Jun	7.6	5-Sep	7
25-Apr	3	18-Jun	15.2	7-Sep	5.4
29-Apr	0.2	1-Jul	0.8	12-Sep	5
3-May	0.4	2-Jul	0.4	13-Sep	34
4-May	2.4	6-Jul	0.6	14-Sep	0.6
5-May	6.2	7-Jul	0.8		
15-May	3.4	13-Jul	2		

2.2.2. Plant protection products applied

All the active ingredients used in the upper Meolo basin in the growing season 2008 were identified and the amounts applied were estimated from sales data and through interview with the main farmers. In this work only the organic chemicals have been considered. In the 2008 season 34 organic active ingredients were applied (3429 kg). Only one, S-metolachlor, has been applied on two different crops, maize and soybean. Chemical applications were distributed in the entire season, starting at the end of March with the pre-emergence applications on cereals, identifying application windows and choosing the day in the middle of the window as application date. Also multiple applications patterns were taken into account (table 2.2).
Physical-chemical properties and halflives of the substances were collected using the official data available, even they may be not the best information (table 2.3). Anyway, giving the high number of substances, they were used for the study. For very precise works on a few substances, using more precise data is recommended.

Table 2.2. Active ingredients applied in upper Meolo basin in the productive season 2008, application date, treated area, amount per treatment and application rates (w = wheat, s = soybean, m = maize, v = vine; H = herbicide, F = fungicide, I = insecticide).

Date	Crop	Action	a.i.	Treated area (ha)	Amount (kg)	Application Rate (kg/ha)
23-mar	w	H	florasulam	18.5	0.07	0.004
23-mar	w	H	fluroxypyr	18.5	2.8	0.15
05-apr	m	H	acetochlor	80.4	152.8	1.90
05-apr	m	H	dichlormid	80.4	25.48	0.32
05-apr	m	H	isoxaflutole	134	7.04	0.05
05-apr	m	H	pendimethalin	110.55	10.30	0.09
05-apr	m	H	S-metolachlor	254.6	318.13	1.25
05-apr	m	H	terbuthylazine	365.15	234.35	0.64
15-apr	v	H	glyphosate	184.3	66.36	0.36
23-apr	v	F	mancozeb	70	140	2
23-apr	s	H	metribuzin	37	6.3	0.17
23-apr	s	H	S-metolachlor	21	24	1.14
26-apr	v	F	mancozeb	134.1	236	1.76
26-apr	v	F	metiram	14.9	26.70	1.79
02-mag	v	F	mancozeb	70	140	2
09-mag	v	F	mancozeb	134.1	236	1.76
09-mag	v	F	metiram	14.9	26.70	1.79
12-mag	v	F	mancozeb	70	140	2
26-mag	v	F	dimethomorph	124.5	22.41	0.18
26-mag	v	F	fosetyl-aluminum	14.9	12.12	0.81
26-mag	v	F	iprovalicarb	94.7	12.10	0.13
26-mag	v	F	mancozeb	209.2	258.14	1.23
26-mag	v	F	metalaxyl-M	29.8	1.65	0.06
26-mag	v	F	sulfur	194.3	512.75	2.64
30-mag	v	H	glyphosate	184.3	66.36	0.36
03-giu	w	F	azoxystrobin	63.2	12.75	0.20
03-giu	s	H	cycloxydim	56	13.4	0.24
03-giu	v	F	dimethomorph	124.5	22.41	0.18
03-giu	s	H	imazamox	69	3.32	0.05
03-giu	v	F	mancozeb	124.5	149.4	1.2
03-giu	v	F	penconazole	29.8	0.90	0.03
03-giu	w	F	procloraz	33.6	13.6	0.40
03-giu	w	F	propiconazole	33.6	3.06	0.09
03-giu	v	F	sulfur	194.3	512.75	2.64

Table 2.2 – continued.

Date	Crop	Action	a.i.	Treated area (ha)	Amount (kg)	Application Rate (kg/ha)
03-giu	s	H	thifensulfuron-methyl	60	0.23	0.004
10-giu	v	F	fosetyl-aluminum	14.9	12.12	0.81
10-giu	v	F	iprovalicarb	94.7	12.10	0.13
10-giu	v	F	mancozeb	84.7	108.74	1.28
10-giu	v	F	metalaxyl-M	29.8	1.65	0.06
15-giu	v	F	dimethomorph	124.5	22.41	0.18
15-giu	v	F	mancozeb	124.5	149.4	1.2
15-giu	v	F	penconazole	29.8	0.90	0.03
15-giu	v	F	sulfur	194.3	512.75	2.64
19-giu	v	I	flufenoxuron	44.7	2.25	0.05
23-giu	v	F	fosetyl-aluminum	14.9	12.12	0.81
23-giu	v	F	iprovalicarb	94.7	12.10	0.13
23-giu	v	F	mancozeb	84.7	108.74	1.28
23-giu	v	F	metalaxyl-M	29.8	1.65	0.06
26-giu	v	F	dimethomorph	124.5	22.41	0.18
26-giu	v	F	mancozeb	124.5	149.4	1.2
26-giu	v	F	meptyldinocap	14.9	2.63	0.18
26-giu	v	F	penconazole	29.8	0.90	0.03
30-giu	m	H	dicamba	107.2	26.09	0.24
30-giu	v	F	dimethomorph	189.2	34.08	0.18
30-giu	m	H	mesotrione	80.4	6.4	0.08
30-giu	m	H	nicosulfuron	113.9	4.56	0.04
09-lug	v	F	meptyldinocap	14.9	2.63	0.18
11-lug	v	I	chlorpyrifos	104.3	52.17	0.50
26-lug	v	F	cyprodinil	44.73	15.365	0.34
26-lug	v	F	fludioxonil	59.7	10.25	0.17
26-lug	v	F	pyrimethanil	144.5	100.80	0.70
09-ago	v	I	chlorpyrifos	104.3	26.08	0.25
09-ago	v	F	pyrimethanil	144.5	78.40	0.54
14-ago	v	H	glyphosate	104.3	37.56	0.36
14-ago	v	H	glufosinate-ammonium	59.8	18	0.30
20-ago	v	F	fenhexamid	24.9	12.5	0.50

Table 2.3. Physical-chemical properties and halflives of the substances used in upper Meolo basin in the productive season 2008. Where more than one value was proposed by authors, the field value giving a worst case was chosen. (Italic: herbicides, bold: insecticides; normal: fungicides).

Active Ingredient	MW	WS (g/m^3)	VP (Pa)	Log K_{OW}	Log K_{OC} (L/kg) or K_d	DT_{50} soil (d)
acetochlor	268.8	223[(1)]	6.00E-03[(1)]	4.14[(1)]	2.19[(2)]	12.1[(2)]
azoxystrobin	403.4	6[(1)]	1.10E-10[(1)]	2.5[(3)]	2.29[(3)]	200[(3)]
chlorpyrifos	350.6	1.4[(1)]	2.70E-03[(1)]	5.261[(3)]	4.13[(3)]	70[(3)]
cycloxydim	325.5	53[(1)]	1.00E-05[(1)]	1.36[(3)]	1.15[(3)]	1[(3)]
cyprodinil	225.3	13[(1)]	5.10E-04[(2)]	4[(3)]	3.79[(3)]	40[(3)]
dicamba	221.0	6100[(1)]	1.67E-03[(1)]	0.55[(3)]	0.34[(3)]	10[(3)]
dichlormid	208.1	5000[(1)]	8.00E-01[(1)]	1.84[(1)]	1.57[(4)]	8[(2)]
dimethomorph	387.9	49.2[(1)]	9.85E-07[(2)]	2.63[(3)]	2.63[(3)]	44[(3)]
fenhexamid	302.2	20[(1)]	4.00E-07[(1)]	3.51[(1)]	2.68[(2)]	1[(2)]
florasulam	359.3	6360[(1)]	1.00E-05[(1)]	-1.22[(1)]	1.34[(2)]	8.5[(2)]
fludioxonil	248.2	1.8[(1)]	3.90E-07[(1)]	4.12[(3)]	3.91[(3)]	18[(2)]
flufenoxuron	488.8	0.00152[(1)]	6.52E-12[(1)]	3.9[(3)]	3.69[(3)]	42[(3)]
fluroxypyr	255.0	6500[(2)]	3.78E-09[(1)]	-1.24[(1)]	1.82[(2)]	51[(3)]
fosetyl-aluminum	354.1	111300[(1)]	1.00E-07[(1)]	-2.1[(3)]	(Kd) 46[(3)]	1[(3)]
glufosinate-ammonium	198.2	1370000[(1)]	3.10E-05[(2)]	< 0.1[(1)]	(Kd) 115[(3)]	18[(3)]
glyphosate	169.1	10500[(1)]	1.31E-05[(1)]	-3.2[(2)]	(Kd) 350[(3)]	60[(3)]
imazamox	305.3	4160[(1)]	1.33E-05[(2)]	0.7[(3)]	0.49[(3)]	41[(3)]
iprovalicarb	320.4	8.9[(1)]	7.70E-08[(1)]	3.2[(1)]	2.03[(2)]	15.5[(2)]
isoxaflutole	359.3	6.2[(1)]	1.00E-06[(1)]	2.32[(3)]	2.11[(3)]	20[(3)]
mancozeb	271.3	6.2[(1)]	1.30E-05[(2)]	0.26[(1)]	(Kd) 10[(3)]	0.5[(3)]
meptyldinocap	364.4	0.248[(2)]	7.92E-07[(2)]	6.55[(2)]	4.77[(2)]	15[(2)]
mesotrione	339.3	15000[(1)]	5.69E-06[(1)]	0.11[(3)]	2.04[(3)]	15[(3)]
metalaxyl-M	279.3	26000[(1)]	3.30E-03[(1)]	1.71[(1)]	2.82[(2)]	39[(3)]
metiram	1089	2[(2)]	1.00E-05[(2)]	1.76[(3)]	1.8[(3)]	2.7[(3)]
metribuzin	214.3	1050[(1)]	5.80E-05[(1)]	1.6[(3)]	1.38[(3)]	45[(3)]
nicosulfuron	410.4	70[(1)]	8.00E-10[(2)]	-1.7[(3)]	1.4[(3)]	26[(3)]
penconazole	284.2	73[(1)]	1.70E-04[(1)]	3.72[(3)]	3.51[(3)]	133[(3)]
pendimethalin	281.3	0.33[(1)]	1.94E-03[(1)]	5.2[(1)]	4.20[(2)]	90[(2)]
procloraz	376.7	34.4[(1)]	9.00E-05[(1)]	4.12[(1)]	3.70[(4)]	270[(4)]
propiconazole	342.2	100[(1)]	2.70E-06[(1)]	3.72[(3)]	3.51[(3)]	110[(3)]
pyrimethanil	199.3	121[(1)]	2.20E-03[(1)]	2.84[(3)]	2.7[(3)]	30[(3)]
S-metolachlor	283.8	480[(1)]	3.70E-03[(1)]	3.05[(1)]	2.29[(4)]	22[(2)]
terbuthylazine	229.7	8.5[(1)]	1.50E-04[(1)]	2.88[(3)]	2.44[(3)]	60[(3)]
thifensulfuron-methyl	387.4	2240[(1)]	1.70E-08[(1)]	-1.7[(3)]	1.65[(3)]	10[(3)]

(1) Tomlin, 2003; (2) FOOTPRINT, 2006; (3) Verro *et al.*, 2009a; (4) Agritox website

2.2.3. Exposure assessment procedures

Being Meolo a resurgence river, without high variations of water flow among the years, water flow data were estimated by 2004 measurements in a location (Roncade) close to 2008 site (Verro *et al.*, 2009a). To each 2008 date a water flow value has been given, as the nearest water flow value in time assuming the same water flow range between 2004 and 2008 (table 2.4). In case of equal distance from two different values, the lower was chosen as worst case. Rain events have been identified according to rainfall data (table 2.1). For physical-chemical properties of the chemicals see table 2.3. The chemical load to the water body follows both runoff and drift transport patterns.

Table 2.4. Water flows in Roncade station in 2004 (from Verro *et al.*, 2009a) and correspondence between measurement date in 2004 and dates in 2008.

Date	Correspondent dates in 2008	Water Flow (m3/sec)
30-Apr	23-Mar to 2-May	1.90
4-May	3-May to 4-May	2.20
5-May	5-May	2.20
6-May	6-May	2.20
7-May	7-May	2.70
8-May	8-May	2.70
9-May	9-May to 15-May	2.70
22-May	16-May to 27-May	2.20
3-Jun	28-May to 7-Jun	1.60
12-Jun	8-Jun to 12-Jun	2.00
13-Jun	13-Jun to 18-Jun	2.00
25-Jun	19-Jun to 25-Jun	1.20
26-Jun	26-Jun to 11-Jul	1.20
27-Jul	12-Jul to 30-Jul	1.40
3-Aug	31-Jul to 5-Aug	1.40
7-Aug	6-Aug to 10-Aug	1.60
13-Aug	11-Aug to 17-Aug	1.60
21-Aug	18-Aug to 21-Aug	1.80
22-Aug	22-Aug to 26-Aug	1.60
31-Aug	27-Aug to 7-Sep	1.60
14-Sep	8-Sep to 14-Sep	1.90

To assess the load of chemicals by runoff a first selection was made. The chemicals were divided in three blocks, according to the application volumes.
For the first block (below 10 kg), load was calculated for chemicals with Log K_{OC} < 3 and only for rain events > 20 mm and occurring within 30 days

from the application dates (criterion not applied for chemicals with DT_{50} in soil > 60 d).
For the chemicals of the second block (10 – 20 kg) the selection parameters were the same as the first block, except for rainfall (10 mm).
Among the high amount applied (above 20 kg) active ingredients with Log K_{OC} < 5 and DT_{50} in soil > 7 d were taken into account. Glyphosate was excluded from the procedure because, as a cation, it is highly bound to the inorganic part of the soil.
This is a rough selection, but based on the following principles:

- PECs are strictly related to application volumes;
- chemicals highly bounded to soil particles (estimated with Log K_{OC} or K_d) are poorly moved by runoff;
- runoff amounts are related to the water out-flowed from soil, which is related to rainfall volumes;
- after application, chemicals degrade according to their DT_{50} values, thus time between application and rainfall is an important parameter.

For the selected chemicals SoilFug model (version 1.2, Di Guardo *et al.*, 1994) was applied. SoilFug is a fugacity-based model capable to predict runoff patterns for organic pesticides. The main soil type of the upper Meolo basin is silty loam (ARPAV, 2004), thus the following parameters for this soil scenario were used in SoilFug model: soil depth 0.32 m, diffusive depth 0.16 m, water fraction 0.3 (Finizio *et al.*, 2005), organic carbon fraction 0.005 (as the half of organic matter percentage proposed by worst case for the Italian scenario by FOCUS, 2001). Rainfall data were collected from the San Biagio di Callalta (TV) station (table 2.1) and outflow data were calculated according to Lutz (1984) and Maniak (1982).
The results were diluted according to the water flows previously estimated.

The assessment of the drift load was made starting from the results of Verro *et al.*, (2009a). They assessed the risk in the same area with an extremely accurate procedure related to the spatial information on the crops (maize, soybean, vine and sugar beet). The drift load of 2004 growing season was taken into account and the percentages of drift transportations of the chemicals used in 2004 that have been applied also in 2008 were estimated. For each crop mean values according to action (fungicide, herbicide or insecticide) were also calculated.
Thus to chemicals used in the productive season 2008 a drift value percentage was given according to the previous calculation. For the active ingredients that were applied only in 2008, mean drift value calculated for each crop based on action were used. For active ingredients applied on wheat (not considered in 2004), sugar beet values were used, because the height of the plant to which chemicals are sprayed is very similar.
Herbicides were excluded from the procedure because of their application pattern at the ground level. Drift is not completely avoided but the amounts

that reach the water body due to drift transport are at least one order of magnitude lower than runoff transport, as shown in the results of Verro *et al.*, 2009a. The procedure was made for the two herbicides applied on vineyard, glyphosate and glufosinate-ammonium, which show different properties that bind them to soil particles and make them more susceptible to drift than runoff.
The amounts calculated were diluted according to the water flows previously estimated.
This procedure is affected by an error which cannot be calculated due to the fact that spatial information on crops, an important input for the 2004 assessment, were not available for growing season 2008. Anyway, the area is the same and permanent crops (vineyard in this case, for which the major amount of chemicals is used) are thought not to change too much in four years. In contrast, it is not the same for non-permanent crops. On them, however, almost only herbicides are applied, for which the drift load has been assumed as negligible.

2.2.4. Predicting risk for individual chemicals

The characterisation of risk for individual chemicals was performed by applying traditional approaches based on the calculation of toxicity/exposure ratios according to the European directives (EC, 1991; 2003) and on the application of suitable risk indices (Finizio *et al.*, 2001).
Toxicological data on the three organisms conventionally assumed as representative of the aquatic ecosystems (algae, *Daphnia* and fish) are available for most plant protection products currently used in Europe. Table 2.5 reports ecotoxicological data for the plant protection products applied in the upper Meolo river basin.

Table 2.5. Ecotoxicological properties used for aquatic (algae and *Daphnia* EC_{50}s and fish LC_{50}) risk assessment. (italic: herbicides, bolded: insecticides; normal: fungicides).

Active Ingredient	Algae 72 h EC50 (µg/L)	*Daphnia* 48 h EC50 (µg/L)	Fish 96 h LC50 (µg/L)
acetochlor	2.70E-01[(2)]	8.60E+03[(2)]	3.60E+02[(2)]
azoxystrobin	3.60E+02[(3)]	2.30E+02[(3)]	4.90E+02[(3)]
chlorpyrifos	1.20E+03[(3)]	2.00E-01[(3)]	3.00E+00[(3)]
cycloxydim	3.20E+04[(3)]	2.41E+05[(3)]	2.20E+05[(3)]
cyprodinil	5.20E+03[(3)]	3.30E+01[(3)]	2.41E+03[(3)]
dicamba	3.60E+04[(3)]	1.10E+05[(3)]	1.35E+05[(3)]
dichlormid	3.30E+04[(4)]	1.61E+05[(4)]	1.41E+05[(4)]
dimethomorph	2.90E+04[(3)]	4.90E+04[(3)]	3.40E+03[(3)]
fenhexamid	2.61E+04[(4)]	1.88E+04[(4)]	1.34E+03[(4)]
florasulam	8.94E+00[(2)]	2.92E+05[(2)]	1.00E+05[(2)]
fludioxonil	9.30E+02[(3)]	1.10E+03[(3)]	2.30E+02[(3)]
flufenoxuron	4.00E+03[(3)]	4.00E-02[(3)]	4.90E+00[(3)]
fluroxypyr	4.98E+04[(2)]	1.00E+05[(2)]	1.43E+04[(2)]
fosetyl-aluminum	2.19E+04[(3)]	1.89E+05[(3)]	9.43E+04[(3)]
glufosinate-ammonium	1.00E+06[(3)]	5.60E+05[(3)]	5.10E+05[(3)]
glyphosate	4.80E+04[(3)]	2.18E+05[(3)]	3.80E+04[(3)]
imazamox	3.70E+01[(3)]	6.10E+05[(3)]	6.10E+05[(3)]
iprovalicarb	1.00E+04[(2)]	1.98E+04[(2)]	2.27E+04[(2)]
isoxaflutole	1.60E+01[(3)]	6.20E+04[(3)]	6.20E+04[(3)]
mancozeb	3.52E+01[(3)]	7.30E+01[(3)]	7.40E+01[(3)]
meptyldinocap	2.12E+03[(2)]	4.10E+00[(2)]	5.69E+01[(2)]
mesotrione	4.50E+03[(3)]	9.00E+05[(3)]	6.00E+05[(3)]
metalaxyl-M	4.30E+04[(3)]	2.80E+04[(3)]	1.00E+05[(3)]
metiram	3.00E+02[(3)]	1.10E+02[(3)]	3.30E+01[(3)]
metribuzin	4.30E+01[(3)]	4.50E+03[(3)]	7.46E+04[(3)]
nicosulfuron	2.27E+05[(3)]	9.00E+04[(3)]	6.57E+04[(3)]
penconazole	8.30E+02[(3)]	7.00E+03[(3)]	1.70E+03[(3)]
pendimethalin	6.00E+00[(3)]	2.80E+02[(3)]	1.38E+02[(3)]
procloraz	5.50E+00[(2)]	4.30E+03[(2)]	1.50E+03[(2)]
propiconazole	7.60E+02[(2)]	4.80E+03[(2)]	5.30E+03[(2)]
pyrimethanil	1.20E+03[(3)]	2.90E+03[(3)]	1.06E+04[(3)]
S-metolachlor	7.70E+01[(2)]	2.50E+04[(2)]	3.90E+03[(2)]
terbuthylazine	1.58E+01[(3)]	2.10E+04[(3)]	3.80E+03[(3)]
thifensulfuron-methyl	1.59E+01[(3)]	4.70E+05[(3)]	5.00E+05[(3)]

(1) Tomlin, 2003; (2) FOOTPRINT, 2006; (3) Verro *et al.*, 2009a; (4) Agritox website.

Toxicity/Exposure Ratios (TER) were calculated for each substance, for each load event in the river according to equation 2.1.

$$TER = EC_{50}/PEC \quad \text{or} \quad TER = LC_{50}/PEC \qquad \text{(Eq. 2.1)}$$

The ecotoxicological information on the three indicator organisms were put together by using a risk index for the aquatic ecosystem. The PRISW-1 index (Finizio *et al.*, 2001), giving risk scores ranging from 0 to 100, was applied. The structure of the index is shown in table 2.6 and in equation 2.2:

$$PRISW\text{-}1 = (A \times W) + (B \times W) + (C \times W) \qquad \text{(Eq. 2.2)}$$

where:
A, B and C are scores according to PECs for algae, *Daphnia* and fish;
W is a weight assigned according to table 2.6.

Table 2.6. PRISW-1 Risk classification intervals, scores, and weights (W) for non target organisms in surface water system (after Finizio *et al.*, 2001)

ALGAE (A)		**DAPHNIA (B)**		**FISH (C)**	
(EC_{50}/PEC)	SCORE	(EC_{50}/PEC)	SCORE	(LC_{50}/PEC)	SCORE
> 10000	0	> 10000	0	> 10000	0
10000 – 1000	1	10000 – 1000	1	10000 – 1000	1
1000 – 100	2	1000 – 100	2	1000 – 100	2
100 – 10	4	100 – 10	4	100 – 10	4
10 – 2	6	10 – 2	6	10 – 2	6
< 2	8	< 2	8	< 2	8
	W = 3		W = 4		W = 5.5
PRISW-1 = (A × 3) + (B × 4) + (C × 5.5)					

2.2.5. Predicting risk for mixtures

The effect of a mixture of chemicals can be described according to two different models: the Concentration Addition model (CA) by Löewe, applicable to chemicals with the same mode of action and the Independent Action model (IA) by Bliss, applicable to substances with different modes of action (Greco *et al.*, 1992). This second model requires a detailed information on the modes of action of all the substances involved in a mixture. Usually the modes of action of the pesticides are known only for target organisms, while for non-target species this information is not available.
Using the CA model for the prediction of the risk of a mixture usually gives higher results than the IA model. Thus the CA model can be assumed as a conservative worst case in situations when specific information is limited

(Drescher and Boedeker, 1995; Finizio *et al.*, 2005; Junghans *et al.*, 2006). It has been demonstrated that the ratio CA/IA even for complex mixture is, in general, relatively low, and rarely above one order of magnitude. For this reason the CA model can be assumed as a reasonable worst case (Drescher and Boedeker, 1995; Finizio *et al.*, 2005). Moreover, in most of the mixture of pesticides occurring in the environment, 80% of their toxic potency is given by a few chemicals, sometimes even two (Finizio *et al.*, 2005; Verro *et al.*, 2009b). The dominance of a few substances in the toxic potency of a mixture reduces the CA/IA ratio, supporting the use of the simpler CA model for a complex mixture. The CA model is explained through equation 2.3:

$$TU_m = \sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{C_i}{EC_{x,i}} \qquad \text{(Eq. 2.3)}$$

where:
C_i is the actual concentration of the individual chemical *i* in the mixture;
$EC_{x,i}$ is the ecotoxicological end-point (e,g, EC_{50}) of the individual chemical *i*;
TU_i are the toxic units of the individual chemical *i*, i.e. the fraction of the ecotoxicological end-point produced by the individual chemical *i* ($TU_i=C_i/EC_{x,i}$);
TU_m are the toxic units of the mixture.

2.2.6. Sampling scheme

In the sub-basin outlet, samples of water and sediments were collected downstream the applications of plant protection products. Another sampling station on Meolo river was located immediately after the river spring, upstream the majority of crops, as less impacted control site on the same river (figure 2.1). An additional sampling point was located in a reference river, Livenza (section 2.2.1).
Sediments were taken from the upper sediment layer up to 5 cm of depth, up to 2.4 kg each sampling, on the 20th of May 2008 (prior to insecticide applications) and on the 17th of September 2008 (after all the applications and rain events). After collection, samples were frozen and kept at -20°C.
6 L of water were collected at each sampling site. In the station downstream the majority of crops, sampling dates were: 20th May 2008 (pre-application of insecticides), 8th August 2008 (post-application, after a small rain event) 16th August 2008 (post application, after a larger rain event) and 17th September 2008 (at the end of the productive season). In the station upstream water was collected on 20th May 2008 and 17th September 2008. Water was frozen after collection and kept at -20°C.

2.2.7. Ecotoxicological tests with environmental samples

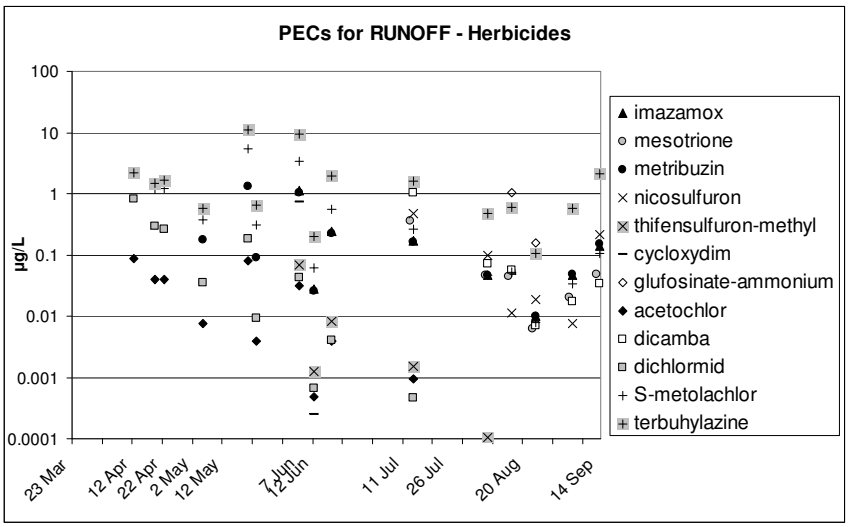
Ecotoxicological test were conducted by the NO MIRACLE partners EKUT and LIMCO at the University of Tübingen, Germany (Langer-Jaesrich and Scheil, personal communication).
Two organisms were tested with the collected sediments: species of the family Chironomidae, that have a sediment dwelling larval stage, and zebrafish (*Danio rerio*). Both the species were kept in an aquarium with the sampled sediments. Chironomids were tested for the following endpoints: larval mortality at different larval stages, locomotive and ventilatory behaviour at different larval stages, stress protein level of L4 larvae, emergence rates, developmental rate (till emergence), sex ratio and ratio of unfit to fit chironomids. Zebrafish was tested for prolonged embryo test, fixation, embedding and analysis of histological samples and stress protein analysis in larvae.
For water only zebrafish was used, with the same endpoints as in sediment tests.

2.3. Results and discussion

2.3.1. Individual chemicals

The PEC for all the organic chemicals applied in upper Meolo basin were calculated following the procedure described in section 2.2.3 and the results for the load due to runoff and drift are shown in figures 2.2, 2.3 and 2.4.

a.



b.

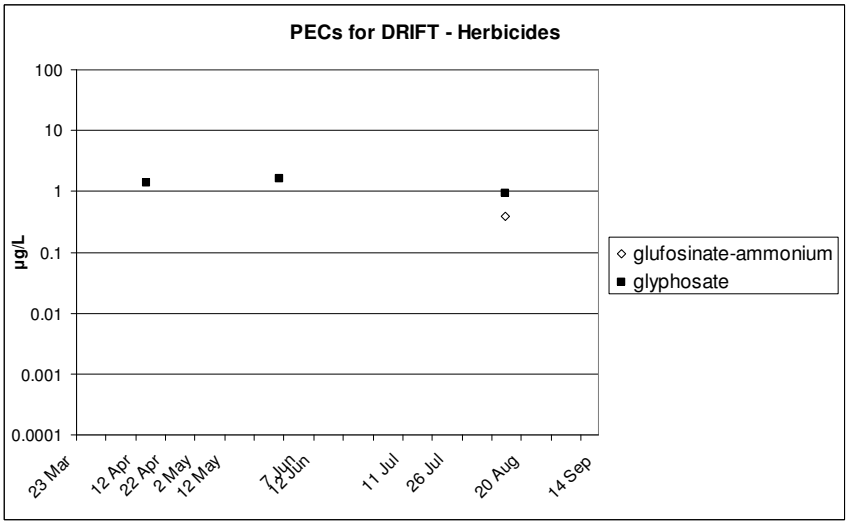
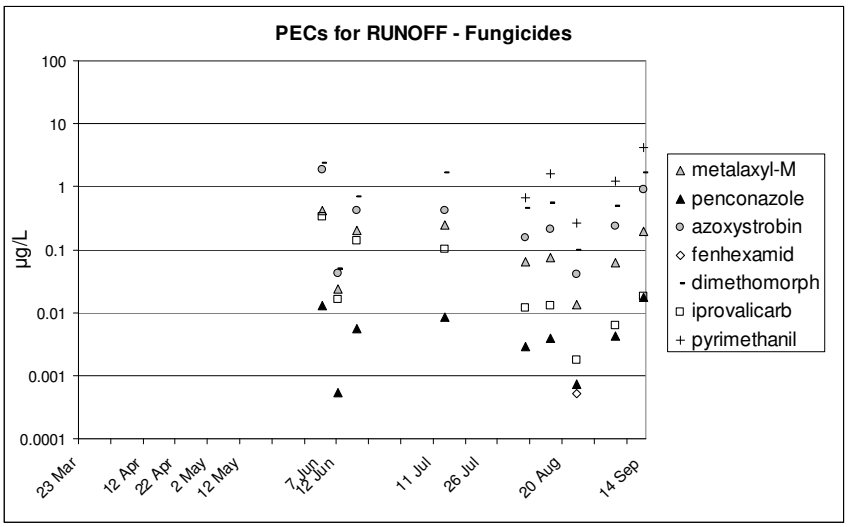


Figure 2.2. Herbicide PECs in upper Meolo basin calculated for runoff (a) and drift (b) events.

a.



b.

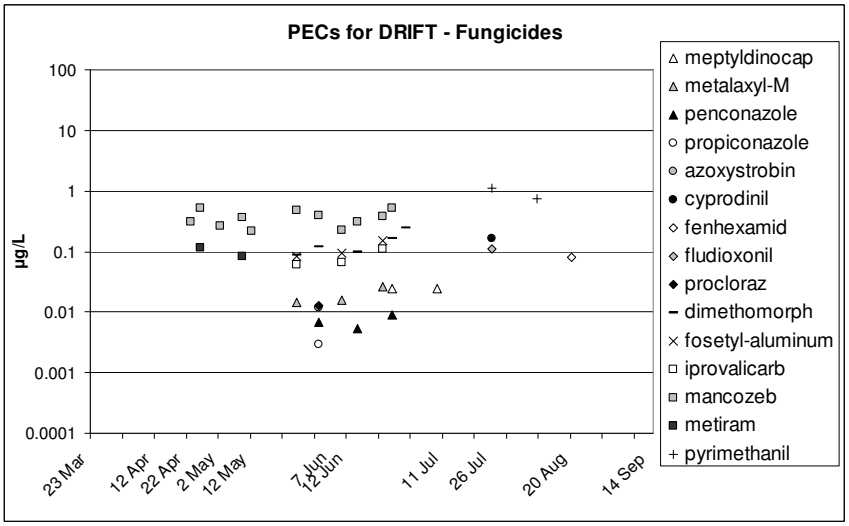
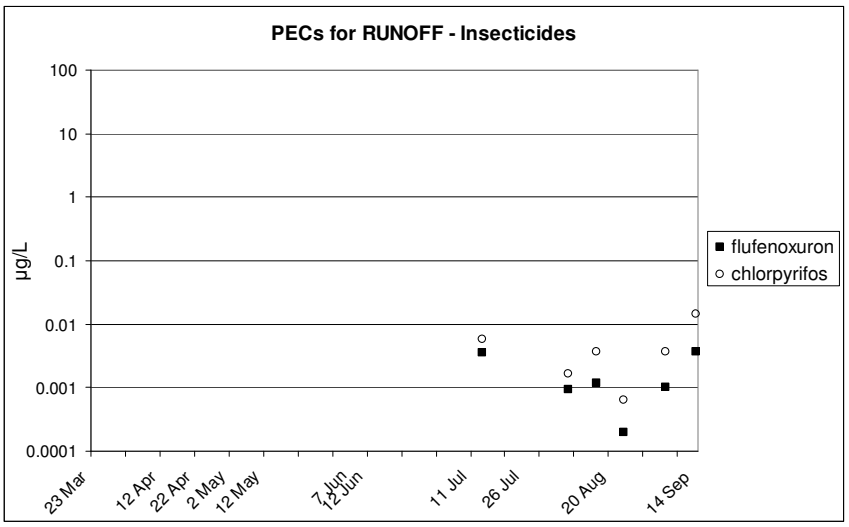


Figure 2.3. Fungicide PECs in upper Meolo basin calculated for runoff (a) and drift (b) events.

a.



b.

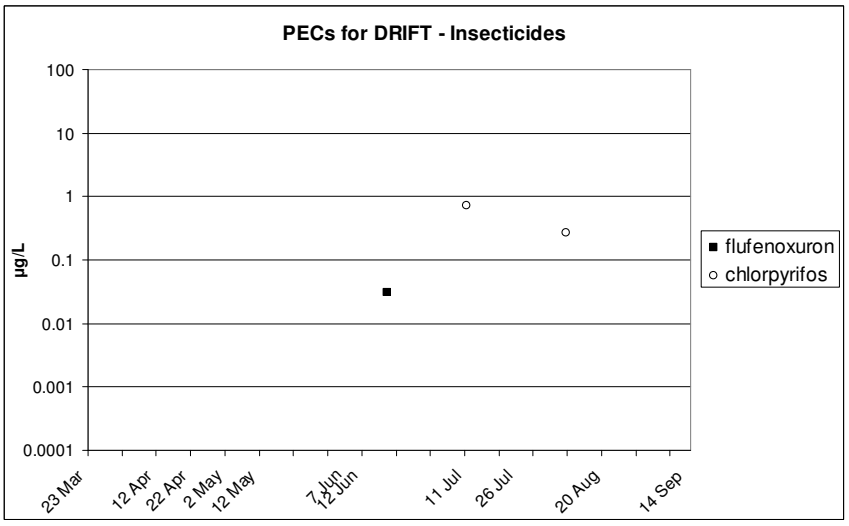
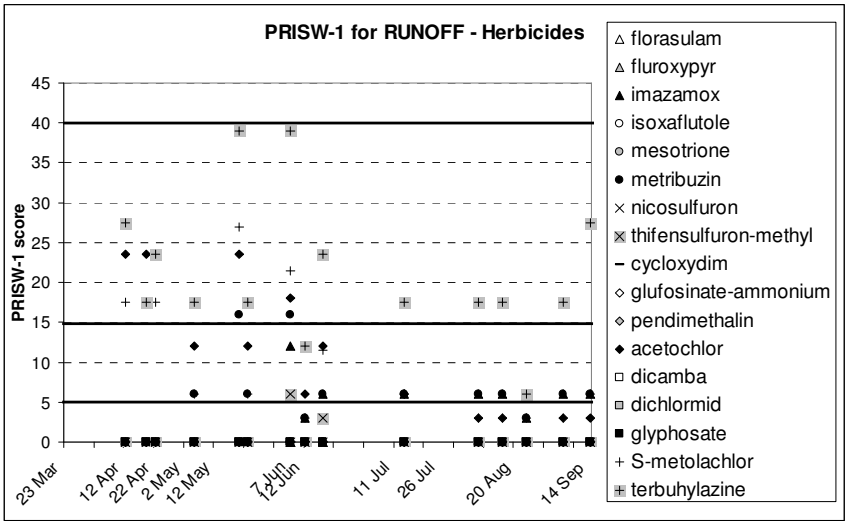


Figure 2.4. Insecticide PECs in upper Meolo basin calculated for runoff (a) and drift (b) events.

Herbicide PECs due to drift (figure 2.2 b) are low or negligible because of the application pattern at the ground level. Only the two herbicides applied to the vineyard are present, with PEC values ranging from 0.40 µg/L for glufosinate-ammonium and 1.62 µg/L for glyphosate. PEC values due to drift for these two herbicides are surprisingly high and for them a refinement of the procedure is advised.
For runoff events (figure 2.2 a) the PECs range from 0.0001 µg/L (thifensulfuron-methyl) to 10.82 µg/L (terbuthylazine). Terbuthylazine and S-metolachlor have the highest values. These are also the herbicides with the highest application amounts, because they are applied on a large area with maize. The application start at the beginning of the productive season, but the load into the river starts after the first rainfall, for runoff, because the contribution due to drift is negligible for most of the herbicides. Although the last applications on the majority of crops (except vineyard) are at the end of June, the load to the river continues until the end of the productive season, because of the properties of the chemicals, that make them available in the soil for the runoff events during summer.
For fungicides PECs are ranging from 0.0005 µg/L (penconazole and fenhexamid) to 4.10 µg/L (pyrimethanil) for runoff events and from 0.003 µg/L (propiconazole) to 1.09 µg/L (pyrimethanil) for drift events. Fungicides are applied only on vineyard and maize, but the two substances applied on maize have low application volumes. The load into the river starts at the first drift event (end of April) and lasts until the end of the productive season for the runoff process. The chemicals with the highest PEC values due to drift (figure 2.3 b) are pyrimethanil and mancozeb. These are the two fungicides with the highest application volumes on vineyards, but, while the first one is present with high amounts in water also due to runoff events, mancozeb is negligible for this process, because its DT_{50} in soil is extremely low (figure 2.3 a).
Only two insecticides are present into the river, both applied on vineyard. Their PEC values range from 0.0002 to 0.014 µg/L for runoff (figure 2.4 a) and from 0.03 to 0.71 µg/L for drift (figure 2.4 b). Always the lower PEC value is for flufenoxuron and the highest for chlorpyrifos. PECs are lower than herbicides (3 orders of magnitude for runoff process and 1 for drift) and fungicides (2 orders of magnitude for runoff and 1 for drift). The load into the river begins with the first application (due to drift) in the second half of June and lasts until the end of the productive season due to the runoff process.

The TERs for algae, *Daphia* and fish, the three organisms used for the risk characterization in the freshwater compartment were calculated. These values were used to calculate the PRISW-1 index, as described in section 2.2.4. Figures from 2.5 to 2.7 show PRISW-1 index values for each chemical (divided according to their action) in each runoff or drift event.

a.



b.

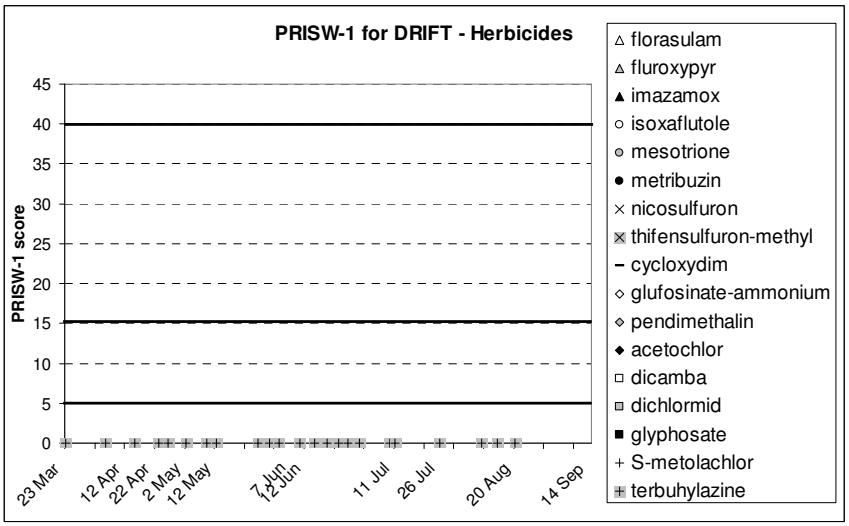
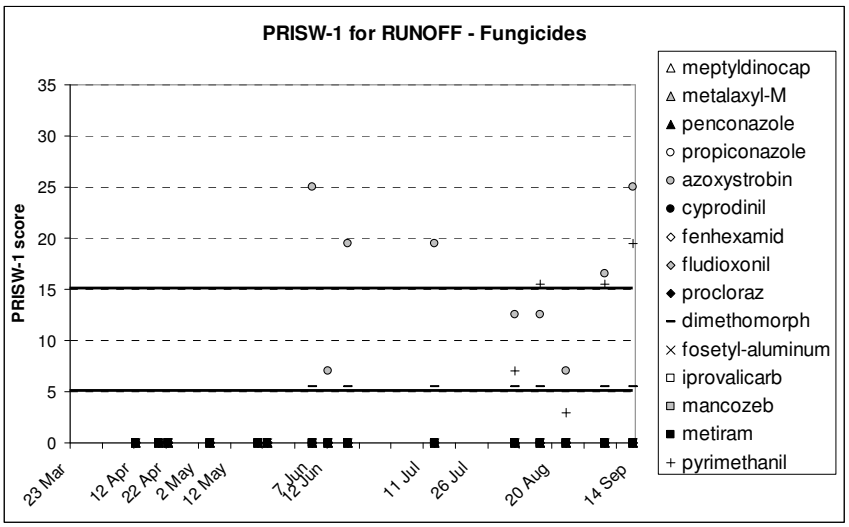


Figure 2.5. PRISW-1 index scores for herbicides, calculated for runoff (a) and drift (b) events. Scores below 5 can be assumed as giving a negligible risk, between 5 and 15 a low risk, between 15 and 40 a medium risk and above 40 a high risk.

a.



b.

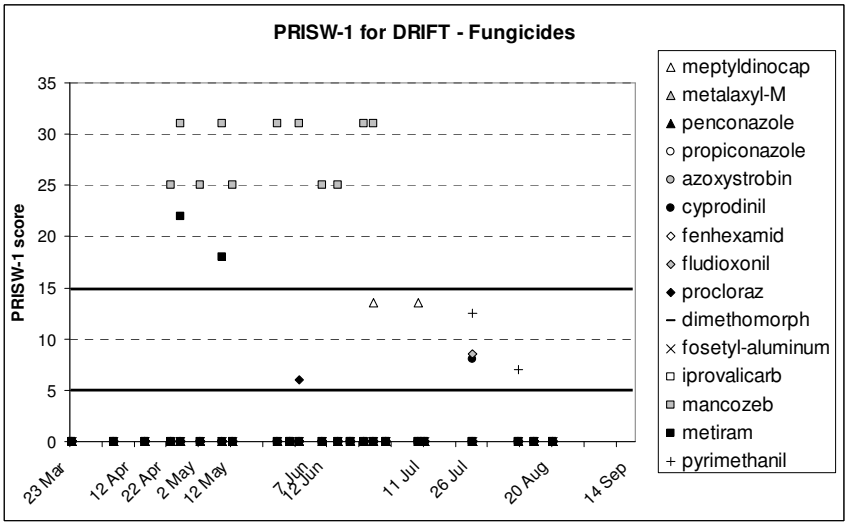
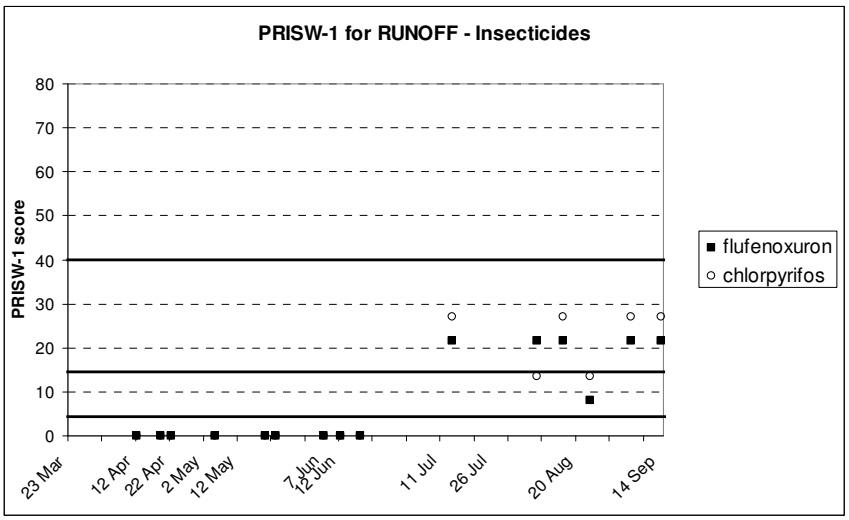


Figure 2.6. PRISW-1 index scores for fungicides, calculated for runoff (a) and drift (b) events. Scores below 5 can be assumed as giving a negligible risk, between 5 and 15 a low risk, and above 15 a medium risk.

a.



b.

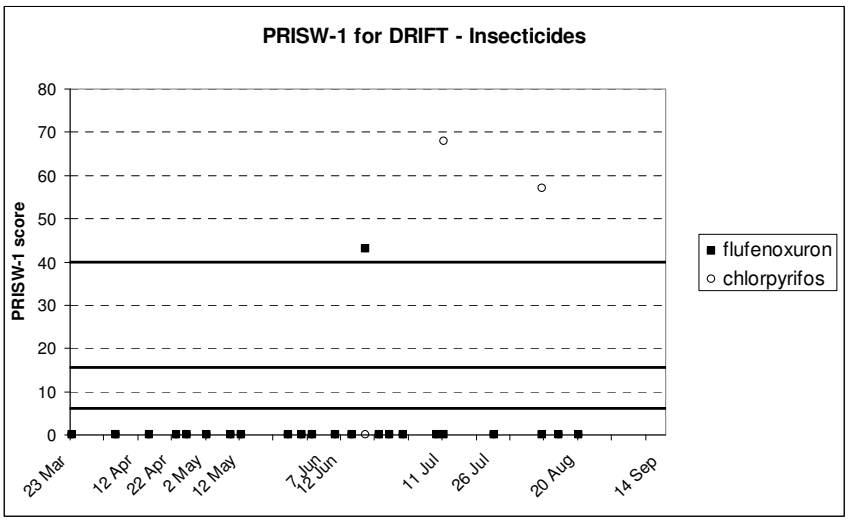


Figure 2.7. PRISW-1 index scores for insecticides, calculated for runoff (a) and drift (b) events. Scores below 5 can be assumed as giving a negligible risk, between 5 and 15 a low risk, between 15 and 40 a medium risk and above 40 a high risk.

The black lines in Figures 2.5, 2.6 and 2.7 represents the thresholds from a risk category to another: chemicals with scores below 5 can be assumed to give a negligible risk, from 5 to 15 a low risk, from 15 to 40 a medium risk and above 40 a high risk (Finizio *et al*., 2001).
Herbicide values ranges from negligible to medium risk for runoff events and are always negligible for drift events. Terbuthylazine has the highest values, the same substance with the highest PECs due to runoff, followed by acetochlor and S-metolachlor. Glyphosate, that is the herbicide with the highest PECs due to drift, gives a negligible risk due to its low toxicity on non target organisms.
Fungicides ranges from negligible to medium risk for both the processes. The chemical with the highest values for runoff is azoxystrobin (due to its toxicity on non target organisms, higher than other fungicides), followed by pyrimethanil, that is also the one with the highest PECs. For drift events mancozeb has the highest values, followed by metiram. The risk due to this two chemicals is higher than the risk caused by pyrimethanil in the drift case.
Insecticides give a medium risk for runoff and high risk for drift (chlorpyrifos higher than flufenoxuron in both cases). The risk is medium/high although PEC values are relatively low, because of the toxicity of insecticides, especially on *Daphnia*.
Overall, the highest risk for runoff process is given by the herbicide terbuthylazine (39: medium) and for drift process by the insecticide chlorpyrifos (68: high).

2.3.2. Mixtures

The risk given by the mixture was predicted following the procedures described in section 2.2.5. The TUs were calculated for algae, *Daphnia* and fish and the time course is shown in figure 2.8.

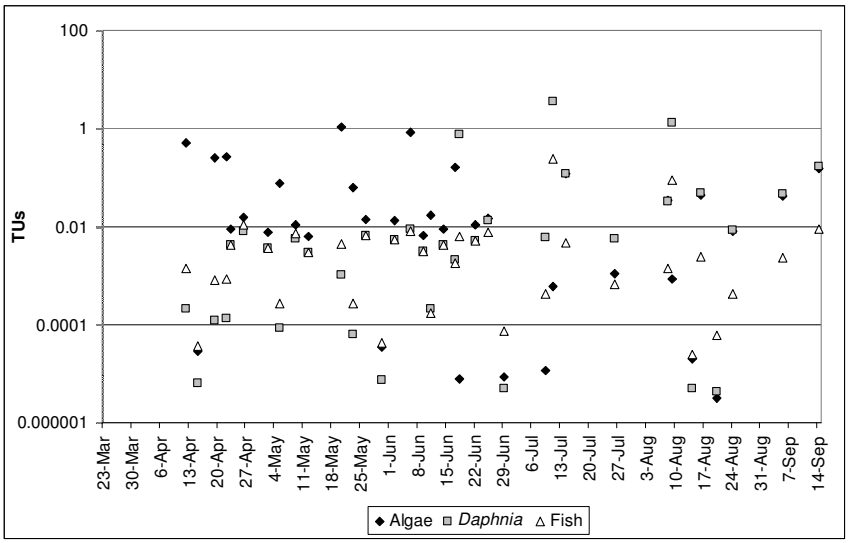


Figure 2.8. Time course of the toxic units for algae, *Daphnia* and fish produced by the mixture composed by all the active ingredients used in upper Meolo basin.

At the beginning of the productive season the risk posed by the mixture is very high for primary producers, due to the use of herbicides, especially on maize. Herbicides are not very toxic for *Daphnia* and fish, thus, the risk for the consumers is lower. In the mid of June and in July the risk for *Daphnia* increases dramatically, consequently to a drift event during the application of an insecticide on vineyard (flufenoxuron). During July, until the end of the prediction, the risk for *Daphnia* is high due to the load of fungicides and insecticides. In the same period the risk for fish is relatively high, but lower than for *Daphnia*, because the sensitivity of fish for these products is lower than those of arthropods. The toxicity of the mixture for the whole period is relatively high, reaching for some dates a value very close or above one TU, indicating a potential for acute toxicity. For algae this happens for two runoff events with a load of terbuthylazine and, secondarily, acetochlor. For *Daphnia* the mixture is more toxic for the drift events after the application of the insecticides (flufenoxuron for the first event, chlorpyrifos for the second and the third) on the vine.

The composition of the mixture is in agreement of what observed by Finizio *et al.* (2005) and Verro *et al.* (2009b): only a few chemicals compose the majority of the mixture. For drift events this is obvious, because the TUs of the mixture derive from the active ingredients applied in that moment (not considered as negligible in our calculations), usually one, sometimes two. The composition of the mixture for runoff events is shown in figure 2.9.

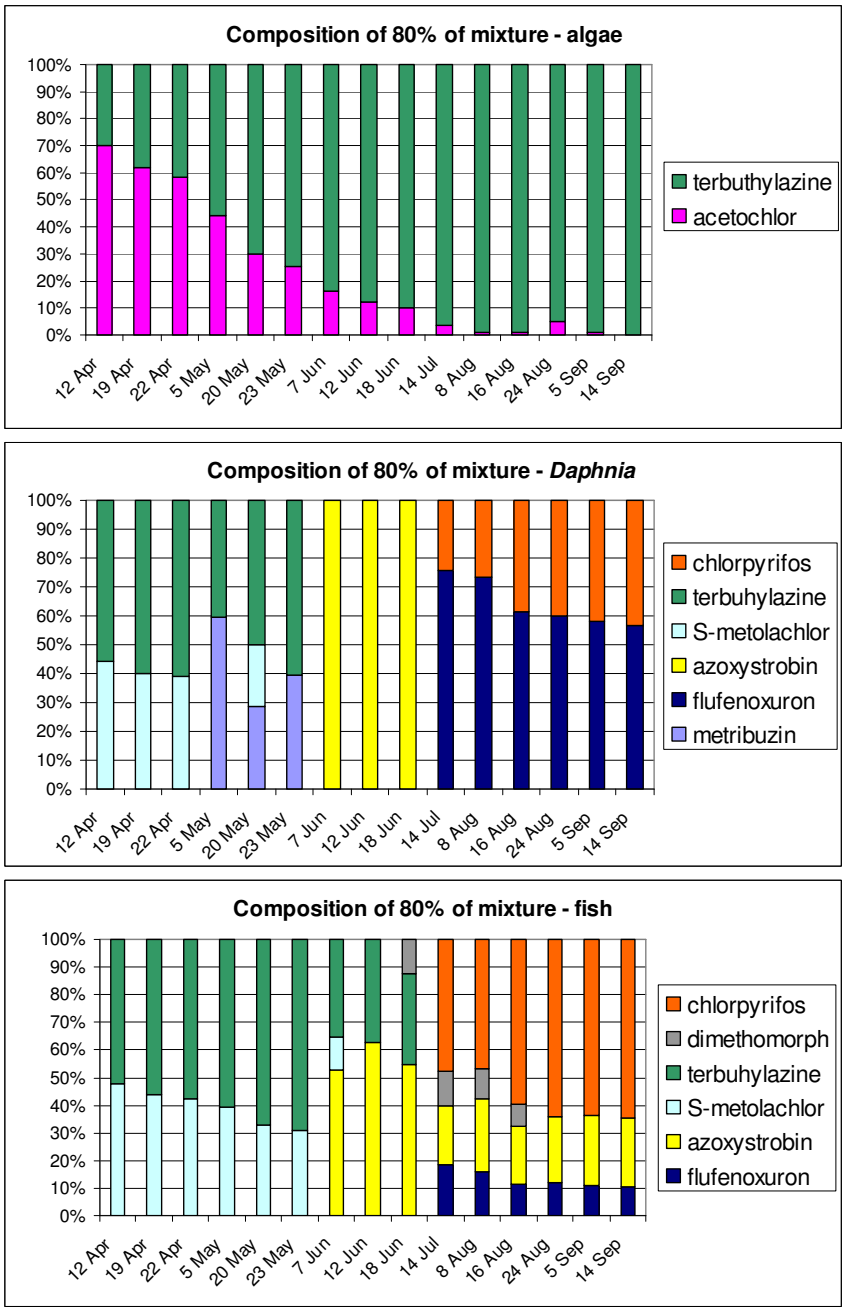


Figure 2.9 The composition of the majority of the mixture due to each runoff event, for algae, *Daphnia* and fish. 80% was taken as trigger value for sake of clarity of the picture.

The risk for algae derives from no more than two compounds for each event: acetochlor and terbuthylazine. The first one is the main compound in the mixture at the beginning of the growing season (April), while from May terbuthylazine becomes more important, remaining until the end of the season as the only chemical in the mixture.
Also the risk for *Daphnia* is given by no more than two substances (only in a runoff event 80% of the mixture is composed by three chemicals). At the beginning of the season they are the herbicides terbuthylazine and S-metolachlor, than the contribution of metribuzin becomes more important. In June the only compound giving the 80% of the mixture potency is the fungicide azoxystrobin, while in July, when the mixture is more toxic for the invertebrates, it is composed almost only by the insecticides flufenoxuron and chlorpyrifos.
For fish the substances composing the mixture are the same than for D*aphnia*, adding the fungicide dimethomorph. The main difference is that the chemicals composing the 80% of the mixture are three or four each event. This is because fish are not the target organisms of herbicides (like algae) or insecticides (like invertebrates), thus there aren't the peaks of toxicity that can be observed for herbicides on algae or for insecticides on *Daphnia*.

The contribution of each crop was assessed by applying the PRISW-1 index to the mixture released from each of them. Originally the index was developed for individual chemicals, but can be applied to a mixture considering the CA approach and calculating the reciprocal of TUs (1/TU=TER), following Verro *et al.*, 2009b. In figure 2.10 the results of the calculation are reported.

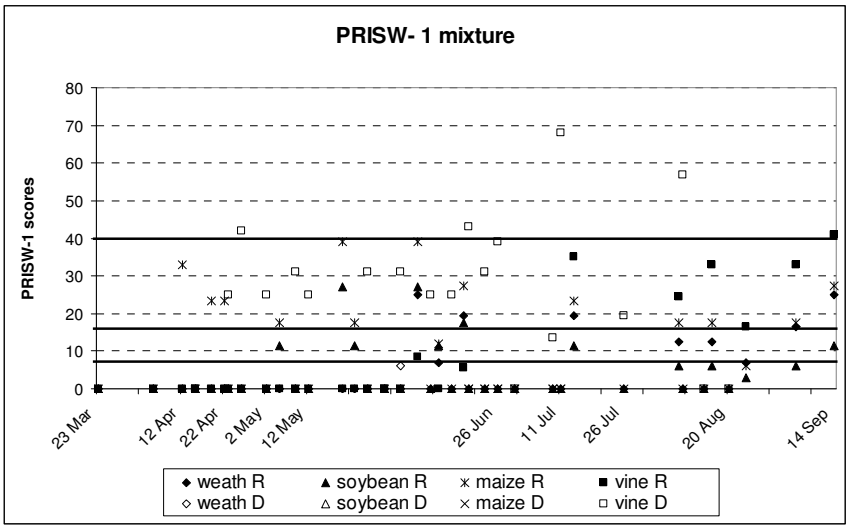


Figure 2.10 PRISW-1 index calculated for the mixture released from each crop (R: runoff events, D: drift events). Scores below 5 can be assumed as giving a negligible risk, between 5 and 15 a low risk, between 15 and 40 a medium risk and above 40 a high risk.

The values are ranging from negligible to high risk, the majority has a medium value. The highest contribution to the mixture risk is given by the drift process for the chemicals applied on vineyard, insecticides and fungicides applied by spraying. Then, medium risk is given by vineyards and maize for runoff process. The contribution of soybean for runoff and of wheat for both the processes is low. Soybean and maize give a negligible risk for drift, because of the application pattern on these two crops.
Overall, the risk given by the drift process is higher than the one given by runoff.

2.3.3. Sediments

Regarding sediments, the Equilibrium Partitioning (EP) approach could be used for assessing exposure. Although the method should be used in a steady state system, Technical Guidance Document (TGD; EC, 2003) addresses it as the method for assessing exposure in sediments also for non steady state systems. Anyway, when experimental data is missing, also the effects would be assessed using EP.
Applying the same method both for exposure and effects would lead to the same risk assessment result of the water compartment.

2.3.4. Ecotoxicological tests

The major results of the tests conducted by NO MIRACLE partners EKUT and LIMCO are reported (Langer-Jaesrich and Scheil, personal communication).

2.3.4.1. Results for sediments

For Chironomidae species all the endpoints reported in section 2.2.7 were tested by Langer-Jaesrich and Scheil (personal communication).
Ten and 17 days after oviposition the survival rate of *Chironomus riparius* larvae were significantly reduced compared to the control, in all samples but in Meolo downstream prior the application of insecticides.
No changes in locomotive or ventilatory activity were recorded for L3 and L4 larvae, in all the samples, 10 and 17 days after oviposition. The same result was achieved for the stress protein level.
The reduction in emergence was significant only in Livenza river in May, but in all the samples a significant early emergence behaviour, and thus a higher developmental rate, was recorded.
Sex and fit/unfit ratios did not change significantly, for all the samples.
The results are schematically summarized in table 2.7.
As a result of the tests, a risk due to the plant protection products load into the River is not confirmed, for chironomids due to the sediment contamination, because the effects were observed only in reference or upstream sediments or in all the samples, never in the downstream case only. Also the presence of the neurotoxic pesticide chlorpyrifos, that should induce behaviour changes (Kienle *et al.*, 2009), did not pose a risk for chironomids.
For zebrafish, histopatological observations were conducted after exposure to the sediments.
In fish exposed to all the sediments but Meolo river upstream September a histopatological effect on liver was observed. Effects on guts were observed for sediments from Livenza and upstream stations in May, on kidneys for Meolo upstream in both the dates and for muscles sediments from Meolo in May (especially downstream) and from Livenza also in both the dates. No effect on pancreas or on larvae stress protein level was observed. The results are summarized in table 2.7.
Also for this organism it seems that the load of plant protection product on sediments did not have an effect on the animals, as seen for chironomids.

2.3.3.2. Results for water

In the tests with water adult zebrafishes were used, with the same histopatological observations after exposure as in the sediment test (Langer-Jaesrich and Scheil, personal communication). All the results are summarized in table 2.7.
Effects on liver were observed for water from Meolo downstream in all the insecticide post-application sampling dates (the 8th and the 16th of August and the 17th of September), while for guts effects were detected in all the sampling dates of the downstream point, also in pre-application. In few organisms of the September water (up- and downstream) and the downstream May water effects on muscles were observed. The structures of kidney and pancreas were unaffected by the exposure to sampled water. No histopatological effects were observed after exposure to water from Livenza river.

In the water tests an indication of a higher effect of the plant protection product load in Meolo river can be seen on adult zebrafish, in liver and gut structures. Although the differences in the semi-quantitative analysis are not significant, there are effects in the downstream samples that are not detected in the upstream or reference ones. This can be assumed as an indication of risk for fish due to the load of pesticides on adults fishes. As shown in figure 2.8 the risk of the mixture is high from August until the end of the productive season, especially for *Daphnia*, but also for fish, with a peak on 9th August.
The stress protein (Hsp 70) level in larvae shows significant differences of August and September samples compared to the control. In Meolo upstream this is true also for the situation in May, while Meolo downstream and Livenza prior the load of insecticides are not significantly different from the control. A clear indication of an effect on larvae due to the pesticide presence does not exist, because all the sampling points show differences from the control at the end of the productive season.

Table 2.7. Summary of the results obtained by EKUT and LIMCO partners in the ecotoxicological tests on chironomids and zebrafish for sediments and water from Meolo and Livenza rivers. Liv: sample from upper Livenza US: sample from Meolo upstream, DS: sample from Meolo downstream; **E**: effect detected, NE: effect not detected, -: sample not collected.

		Sediments						**Water**									
		20-May			17-Sep			20-May			8-Aug		16-Aug		17-Sep		
		Liv	US	DS	Liv	US	DS	Liv	US	DS	US	DS	US	DS	Liv	US	DS
CHIRONOMIDAE	Larval mortality (10 days)	**E**	**E**	NE	**E**	**E**	NE	-	-	-	-	-	-	-	-	-	-
	Larval mortality (17 days)	**E**	**E**	NE	NE	**E**	NE	-	-	-	-	-	-	-	-	-	-
	Locomotive and ventilatory behaviour (10 days)	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
	Locomotive and ventilatory behaviour (17 days)	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
	Stress protein level	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
	Emergence rates	**E**	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
	Developmental rate	**E**	**E**	**E**	**E**	**E**	**E**	-	-	-	-	-	-	-	-	-	-
	Sex ratio	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
	Fit/unfit ratio	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-	-	-	-
ZEBRAFISH	Effects on liver	**E**	**E**	**E**	**E**	NE	**E**	NE	NE	NE	-	**E**	-	**E**	NE	NE	**E**
	Effects on gut	**E**	**E**	NE	NE	NE	NE	NE	NE	**E**	-	**E**	-	**E**	NE	NE	**E**
	Effects on pancreas	NE	NE	NE	NE	NE	NE	NE	NE	NE	-	NE	-	NE	NE	NE	NE
	Effects on kidney	NE	**E**	NE	NE	**E**	NE	NE	NE	NE	-	NE	-	NE	NE	NE	NE
	Effects on muscle	**E**	**E**	**E**	**E**	NE	NE	NE	**E**	NE	-	NE	-	NE	NE	**E**	**E**
	Stress protein level (lavae)	NE	NE	NE	NE	NE	NE	NE	**E**	NE	-	**E**	-	**E**	**E**	**E**	**E**

2.4. Conclusions

The theoretical risk assessment approach applied in this work is based on modelling for assessing exposure and toxicity data from laboratory tests. It indicates high risk for the water compartment. In order to be enough conservative and protective, the procedure uses worst case scenarios and assumptions, even if representative of the real conditions, to describe the actual risk. Moreover, for the same reason, the standard procedures of laboratory toxicity tests are performed applying conditions capable to maximize the effect of tested chemicals (e.g. environmental characteristics capable to ensure the bioavailability of chemicals, etc.).

From the tests performed on water and sediment organisms, the high risk does not seem to be confirmed. Indeed, most of the results do not indicate significant adverse effects produced by the Meolo river samples. For the reasons mentioned above, this is not fully surprising.
However, the tests performed do not reproduce the complexity of natural communities and ecosystems, where the responses to stress may be affected by interactions among several environmental factors and by ecological indirect effects.

In a study on the macrozoobenthos community performed in the same experimental rivers Meolo and Livenza (Bonzini *et al.*, 2008) significant differences were observed in the structure of the communities of the two rivers and it was demonstrated that these differences were determined, at least in part, by plant protection products.

In conclusion, it can be confirmed that the traditional theoretical risk assessment procedures are conservative approaches that tend to overestimate the risk for the sake of environmental protection.
The assessment of the actual site-specific effects on natural communities requires more complex approaches capable to account for the interactions among environmental factors, including combined stressors, as well for the ecological indirect effects.

Acknowledgments

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References

Agritox website: http://www.dive.afssa.fr/agritox/php/fiches.php (accessed in June 2009).
ARPAV (ARPA Veneto), 2004. *Carta dei suoli del bacino scolante in laguna di Venezia*. Agenzia Regionale per la Prevenzione e Protezione dell'Ambiente del Veneto, Padova, Italy, 202 pp.
Bonzini S, Verro R, Otto S, Lazzaro L, Finizio A, Zanin G, Vighi M, 2006. Experimental validation of a GIS-based procedure for predicting pesticide exposure in surface water. *Environl Sci Technol* 40: 7561-7569.
Bonzini S, Finizio A, Berra E, Forcella M, Parenti P, Vighi M, 2008. Effect of river pollution on the colonisation of artificial substrates by macrozoobenthos. *Aquat Toxicol* 89: 1-10.
Di Guardo A, Calamari D, Zanin G, Consalter A, Mackay D, 1994. A fugacity model of pesticide runoff to surface water: development and validation. *Chemosphere* 28: 511-531.
Drescher K, Boedeker W, 1995. Assessment of the combined effects of substances: the relationship between concentration addition and independent action. *Biometrics* 51: 716–730.
EC (European Commission), 1991. Council Directive 91/414/EEC concerning the placing of plant protection products on the market. *Off J Eur Communities* L 1991; 230 19/8/1991.
EC (European Commission), 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in field of water policy. Official Journal of the European Communities, L 327, 22/12/2000.
EC (European Commission), 2003. *Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC for the new notified substances and the Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC on biocides*. Second ed. European Commission, Luxembourg, Luxembourg, Part 1, 2 and 3, 760 pp.
EC (European commission), 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Official Journal of the European Union L 396/1, 30/12/2006.
Feijtel TCJ, Boeije G, Matthies M, Young A, Morris G, Gandolfi C, Hansen B, Fox K, Holt M, Koch V, Schroeder R, Cassani G, Schowanek D, Rosenblom J, Niessen H, 1997. Development of a geo-referenced regional exposure assessment tool for European rivers – GREAT-ER. *Chemosphere* 34: 2351-2374.
Finizio A, Calliera M, Vighi M, 2001. Rating systems for pesticide risk classification on different ecosystems. *Ecotox Environ Saf* 49: 262-274.

Finizio A, Villa S, Vighi M, 2005. Predicting pesticide mixture load in surface waters for a given crop. *Agr Ecosyst Environ* 111: 111-118.
FOCUS, 2001. *FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC*. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.
FOOTPRINT website, 2006. The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.eu-footprint.org/ppdb.html (accessed in June 2009).
Greco W, Unkelbach HD, Pöch G, Sühnel J, Kundi M, Boedeker W, 1992. Consensus on concepts and terminology for combined-action assessment: the Saariselkä agreement. *Arch Complex Environ Stud* 4: 65-72.
Junghans M, Backhaus T, Faust M, Scholze M, Grimme LH, 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquat Toxicol* 76: 93-110.
Kienle C, Kohler HR, Gerhardt A, 2009. Behavioural and developmental toxicity of chlorpyrifos and nickel chloride to zebrafish (*Danio rerio*) embryos and larvae. *Ecotox Environ Saf* 72: 1740-1747.
Lutz W, 1984. *Berechnung von hochwasserabflussen unter anwendung von gebietskenngrossen.* Mittlg. Inst. Hydrologie Wasserwirtschaft, Univ. Karlsruhe, Heft 24. In German.
Maniak U, 1992. Regionalisierung von parametern fur hochwasserabflussganglinier. In: HB Kleeberg (ed.), *Regionalisierung der hydrologie*, DFG, Mittlg. Senatskormm. Fur Wasserf. 11: 325-332. In German.
Sala S and Vighi M, 2008. GIS-based procedure for site-specific risk assessment of pesticides for aquatic ecosystems. *Ecotox Environ Saf* 69:1-12.
Schowanek D, Fox K, Holt M, Boeije G, Vanrollrgehm P, Young A, Morris G, Gandolfi C, Feijtel TCJ, 2004. GREAT-ER: a new tool for management and risk assessment of chemicals in river basins. *Wat Sci Technol* 43: 179-185.
Tomlin, CDS (Ed). *The pesticide manual: a world compendium (13th ed.)*. British Crop Protection Council, Farnham, UK, 1606 pp.
US EPA (Environmental Protection Agency), 1974. Quality criteria for water, Office of water and hazardous materials, US EPA, Washinghton DC, USA.
Verro R, Calliera M, Maffioli G, Auteri D, Sala S, Finizio A, Vighi M, 2002. GIS-based system for surface water risk assessment of agricultural chemicals. 1. methodological approach. *Environ Sci Technol* 36:1532-1538.
Verro R, Finizio A, Otto S, Vighi M, 2009a. Predicting pesticide environmental risk in intensive agricultural areas. I: screening level risk

assessment of individual chemicals in surface waters. *Environ Sci Technol* 43: 522-529.
Verro R, Finizio A, Otto S, Vighi M, 2009b. Predicting pesticide environmental risk in intensive agricultural areas. II: screening level risk assessment of complex mixtures in surface waters. *Environ Sci Technol* 43: 530-537.
Vighi M, Finizio A, Villa S, 2006. The evolution of the environmental quality concept: from the US EPA Red Book to the European Water Framework Directive. *Environ Sci & Pollut Res* 13: 9-14.

CHAPTER III

Theoretical risk assessment for soil compartment: site specific case of a vineyard

Abstract

In agricultural practices a large number of plant protection products are used, and a fraction of them falls onto the soil during the application. Thus soil and its community are exposed to complex mixtures of active ingredients, usually with different chemical and ecotoxicological characteristics, applied on the crops. The transport pattern by which pesticides reach soil is mainly drift, thus the area exposed to agrochemicals is only in the proximities of the crop on which they are applied. Within the NO MIRACLE project a vineyard in Northern Italy was used to perform risk assessment on soil, to be compared with studies on natural communities. Risk was predicted for earthworms for a 14 days period after the application of one of the most toxic compound, chlorpyrifos. Risk for earthworms was relatively low, but they are not the most sensitive species in the soil compartment. The use of alternative organisms in first tier soil risk assessment is discussed. A procedure for mapping risk at larger scale is also proposed.

Keywords: soil, pesticides, earthworms, risk assessment.

3.1 Introduction

In agro-ecosystems several plant protection products (PPP) are used to increase productivity. Their effect, however, is not limited to those organisms for which they have been designed to control, but they can pose a risk also to non-target organisms. The effect is not strictly limited to the treated area, because transport mechanisms can move them towards non-target compartments.
The environmental fate of PPPs is a function of their application patterns, their physical-chemical properties and the environmental characteristics. Some herbicides are usually applied at the ground level, thus they reach the soil compartment directly in the application process. For PPPs sprayed on plants the main transport mechanism to the soil compartment within a field is the direct fallout: during spraying on plants part of the active ingredient (a.i.) is intercepted by leaves and part reaches the soil. Plant interception is a function of crop species and phenological state and is defined as the percentage of retained spray respect to the applied dose (Koch and Weisser, 2001). Harmonized crop interception values for crops are reported by FOCUS (2001).
During their application on crops, chemicals can be transported outside a field by drift. Drift can be defined as the proportion of a product that come directly from nozzles and is transported out of treated field due to air flowed during an application (Collemback, 1982; Hilbert, 1992). Usually, three types of drift can be identified: thermal drift, when lighter droplets are transported to high altitude, vapour drift, after volatilisation from the target, and droplets drift, when wind pushes droplets off-target (Vicari *et al.*, 2001). Studies on drift process have been made by Ganzelmeier *et al.* (1995) and Rautmann (2001) and the results of these works are currently used in PPP registration procedures in EU (Wang and Rautmann, 2008). The exposure due to drift is relevant only in the area closest to the treated field. Indeed equations to calculate drift percentages take into account an exponential decrease (Barmaz, 2009). Measurements of chlorpyrifos few hours after an application on a vineyard indicate that soil concentration 4 meters outside a field is one order of magnitude less than within the field (Barmaz, 2009).
Figure 3.1 shows the transport patterns related to the soil compartment of PPPs applied on a crop.

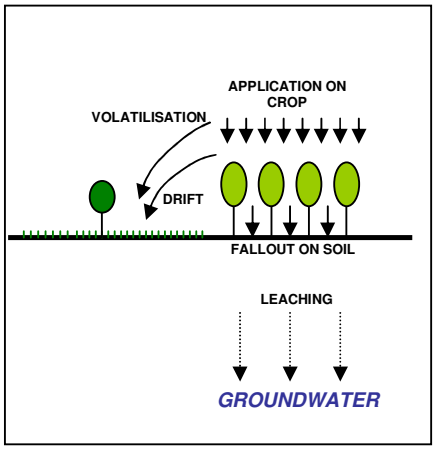

Figure 3.1. Transport patterns related to the soil compartment of PPPs applied on a crop.

Current legislations, Directive 91/414/EEC (EC, 1991) and Technical Guidance Document (TGD) on risk assessment (Vol. II) (EC, 2003), indicate procedures for risk assessment on non-target organisms by comparing the exposure, due to direct fallout or drift, with effect indicators on organisms. The result is an ETR (Exposure/Toxicity Ratio).
The traditional soil organism used for terrestrial risk assessment is earthworm, which is used in the first tier risk assessment of a substance, performed by calculating a Toxicity/Exposure Ratio (TER) (EC, 2002a). Other soil organisms, as gamasid mites or springtails (Collembola), are taken into account just in higher tier risk assessment for persistent substances, performed through standardized tests (for springtail, ISO 11267; ISO, 1999) or proposed methods (for the gamasid mite *Hypoaspis aculeifer*, Løkke and van Gestel, 1998; Bakker *et al.*, 2002) (EC, 2002a).

The work presented in this chapter was done within a master case performed for the NO MIRACLE (Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe) project of the European Commission. A site was identified and used for both aquatic (see chapter II) and terrestrial activities. The main purpose of the master case was the intersection of the theoretical risk assessment with activities on biological communities, both in lab and in field, to observe the real effects given by PPPs application.

3.2. Materials and methods

3.2.1. Site description

The activities were performed, starting from June 2008 until June 2009, in a 5 ha vineyard located in Veneto region (Northern Italy), in Meolo river basin, the same river used for NO MIRACLE master case on the water compartment (see section II).
Three sampling stations were identified: one within the field (A), one 4 m and one 10 m away from the last row (respectively B and C). In the selected vineyard different cultivars are present, with different crop characteristics: A and B points are located inside and near a part of the vineyard with old and tall Pinot Grigio variety, while C point is located near young and small Prosecco variety (figure 3.2).

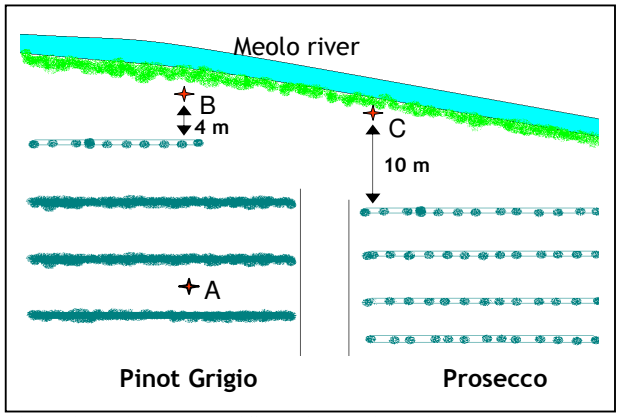

Figure 3.2. Field site scheme (modified after Vaj *et al.*, 2010).

Meteorological data were provided by a meteorological station located 1.5 km from the field site (for rain see table 3.1).

Table 3.1. Rainfall measured from April to September 2008 and from April to June 2009 by the meteorological station located in San Biagio di Callalta (TV).

Date	Rain (mm)	Date	Rain (mm)	Date	Rain (mm)
5-Apr-08	0.2	11-Jun-08	7	16-Apr-09	2.2
6-Apr-08	0.8	12-Jun-08	0.4	17-Apr-09	0.4
9-Apr-08	0.2	14-Jun-08	4	19-Apr-09	2.2
10-Apr-08	2.8	17-Jun-08	7.6	20-Apr-09	5.8
11-Apr-08	6	18-Jun-08	15.2	21-Apr-09	1
12-Apr-08	3.8	1-Jul-08	0.8	23-Apr-09	2.2
13-Apr-08	0.2	2-Jul-08	0.4	26-Apr-09	4.4
15-Apr-08	1.2	6-Jul-08	0.6	27-Apr-09	13.2
16-Apr-08	0.8	7-Jul-08	0.8	28-Apr-09	31
18-Apr-08	6.8	13-Jul-08	2	29-Apr-09	8.8
19-Apr-08	4.6	14-Jul-08	17.8	4-May-09	3.8
21-Apr-08	11.4	17-Jul-08	0.4	5-May-09	7.6
22-Apr-08	0.4	18-Jul-08	2.4	24-May-09	1
25-Apr-08	3	22-Jul-08	0.8	27-May-09	20.6
29-Apr-08	0.2	28-Jul-08	2.2	5-Jun-09	0.2
3-May-08	0.4	8-Aug-08	13.4	6-Jun-09	1.6
4-May-08	2.4	15-Aug-08	2.4	7-Jun-09	7.8
5-May-08	6.2	16-Aug-08	13.6	8-Jun-09	0.2
15-May-08	3.4	23-Aug-08	6.8	9-Jun-09	0.2
18-May-08	49.2	24-Aug-08	0.4	15-Jun-09	4
19-May-08	0.4	2-Sep-08	1.2	20-Jun-09	19.8
20-May-08	1.2	4-Sep-08	12.2	23-Jun-09	0.2
23-May-08	12.2	5-Sep-08	7	24-Jun-09	4.4
25-May-08	1	7-Sep-08	5.4	25-Jun-09	5
4-Jun-08	16	12-Sep-08	5	26-Jun-09	0.2
5-Jun-08	26.8	13-Sep-08	34	27-Jun-09	49.6
6-Jun-08	0.8	14-Sep-08	0.6	28-Jun-09	1.4
7-Jun-08	0.6			29-Jun-09	0.2

3.2.2. Plant protection products applied

All the information about active ingredients used, their amount and the application dates were provided in detail by the farmer. The chemicals applied and their application rates are listed in table 3.2. Official available data on their physical-chemical properties and halflives are reported in table 3.3.

Table 3.2. Active ingredients applied in the vineyard (in italics those applied only only on Pinot Grigio variety) and application rates. H: herbicide, F: fungicide, I: insecticide.

Date	Action	Active ingredient	Application rate (kg/ha)
28-apr-08	F	mancozeb	2.25
28-apr-08	F	sulfur	3.2
5-May-08	F	sulfur	3.2
5-May-08	F	mancozeb	2.25
14-May-08	F	mancozeb	2.25
14-May-08	F	sulfur	3.2
19-May-08	F	sulfur	3.2
19-May-08	F	mancozeb	2.25
19-May-08	F	dimethomorph	0.25
20-May-08	H	glyphosate	0.72
20-May-08	H	oxadiazon	0.74
24-May-08	F	dimethomorph	0.25
24-May-08	F	folpet	1.6
24-May-08	F	mancozeb	1.5
24-May-08	F	sulfur	4
27-May-08	F	sulfur	4
27-May-08	F	folpet	2
2-Jun-08	F	folpet	4
2-Jun-08	F	dimethomorph	0.25
2-Jun-08	F	sulfur	4.8
8-Jun-08	F	mancozeb	2.25
8-Jun-08	F	folpet	1.6
8-Jun-08	F	sulfur	4
14-Jun-08	F	sulfur	4
14-Jun-08	F	folpet	1.6
14-Jun-08	F	mancozeb	1.5
14-Jun-08	F	iprovalicarb	0.15
17-Jun-08	F	*cyprodinil*	0.375
17-Jun-08	F	*fludioxonil*	0.25
20-Jun-08	F	mancozeb	2.25
20-Jun-08	F	sulfur	4
23-Jun-08	H	glyphosate	0.72
27-Jun-08	F	sulfur	4.8
27-Jun-08	F	dimethomorph	0.25
27-Jun-08	I	thiamethoxam	0.05
27-Jun-08	F	copper oxychloride	1.05
5-Jul-08	F	copper oxychloride	1.225
5-Jul-08	F	sulfur	4.8
15-Jul-08	F	sulfur	4.8

Table 3.2 – continued.

Date	Action	Active ingredient	Application rate (kg/ha)
15-Jul-08	F	copper oxychloride	1.4
15-Jul-08	I	chlorpyrifos	0.45
22-Jul-08	F	copper sulphate	0.65
22-Jul-08	F	sulfur	6
30-Jul-08	F	copper sulphate	0.65
30-Jul-08	F	sulfur	6
5-Aug-08	F	*mepanipyrim*	0.5
5-Aug-08	H	glyphosate	0.889
5-Aug-08	F	copper oxychloride	1.125
5-Aug-08	F	sulfur	4.8
11-Aug-08	F	copper sulphate	0.488
11-Aug-08	F	sulfur	4
15-Apr-09	F	folpet	1.2
15-Apr-09	F	sulfur	2.4
20-Apr-09	H	glyphosate	0.72
25-Apr-09	F	folpet	1
25-Apr-09	F	sulfur	2.4
2-May-09	F	sulfur	2.4
2-May-09	F	folpet	1
10-May-09	F	folpet	1
10-May-09	F	sulfur	3.2
16-May-09	F	sulfur	3.2
16-May-09	F	folpet	0.8
22-May-09	F	folpet	0.8
22-May-09	F	sulfur	3.2
29-May-09	F	sulfur	4
29-May-09	F	folpet	0.96
7-Jun-09	F	folpet	1.2
7-Jun-09	F	sulfur	4.4
7-Jun-09	F	*thiophanate-metyl*	0.45
12-Jun-09	F	*cyprodinil*	0.375
12-Jun-09	F	*fludioxonil*	0.25
12-Jun-09	F	folpet	1.029
12-Jun-09	F	copper oxychloride	0.6125
12-Jun-09	F	sulfur	4.4
14-Jun-09	H	glyphosate	0.72
22-Jun-09	I	thiamethoxam	0.05
22-Jun-09	F	copper oxychloride	1.125
22-Jun-09	F	sulfur	4.8

Table 3.3. Physical-chemical properties and halflives used for soil exposure assessment in the vineyard (- = no data available). Where more than one value were proposed by authors, the most conservative field value was chosen. H: herbicide, F: fungicide, I: insecticide.

Active Ingredient	Action	MW	S (g/m3)	VP (Pa)	Log K_{OW}	DT_{50} soil (d)	DT_{50} photo-degradation (d)
carbendazim	F	191.2[(1)]	8[(1)]	1.50E-04[(1)]	1.51[(1)]	10[(2)]	-
chlorpyrifos	I	350.6[(1)]	1.4[(1)]	2.70E-03[(1)]	5.261[(3)]	70[(3)]	5[(4)]
cyprodinil	F	225.3[(1)]	13[(1)]	5.10E-04[(2)]	4[(3)]	40[(3)]	0.24[(5)]
dimethomorph	F	387.9[(1)]	49.2[(1)]	9.85E-07[(2)]	2.63[(3)]	44[(3)]	-
fludioxonil	F	248.2[(1)]	1.8[(1)]	3.90E-07[(1)]	4.12[(3)]	18[(2)]	0.125[(5)]
folpet	F	296.6[(1)]	0.8[(1)]	2.10E-05[(1)]	3.63[(3)]	4.3[(3)]	-
glyphosate	H	169.1[(1)]	10500[(1)]	1.31E-05[(1)]	-3.2[(2)]	60[(3)]	-
iprovalicarb	F	320.4[(1)]	8.9[(1)]	7.70E-08[(1)]	3.2[(1)]	15.5[(2)]	62[(6)]
mancozeb	F	271.3[(1)]	6.2[(1)]	1.30E-05[(2)]	0.26[(1)]	0.5[(3)]	
mepanipyrim	F	222.3[(1)]	3.1[(1)]	2.32E-05[(1)]	3.28[(1)]	57[(2)]	-
oxadiazon	H	345.2[(1)]	1[(1)]	1.00E-04[(1)]	4.91[(1)]	151[(2)]	-
thiophanate-methyl	F	342.4[(1)]	20[(1)]	9.50E-06[(1)]	1.5[(1)]	[5(2)]	-
thiamethoxam	I	291.7[(1)]	4100[(1)]	6.60E-09[(1)]	-0.13[(1)]	39[(2)]	10000[(7)]
copper oxychloride	F	427.1[(1)]	1.19[(2)]	1.00E-08[(2)]	-	10000[(2)]	-
copper sulphate	F	249.7[(1)]	230500[(1)]	3.40E-13[(2)]	-	1600[(2)]	-
sulfur	F	32.1[(1)]	0.063[(2)]	9.80E-05[(2)]	-	30[(2)]	-

(1) Tomlin, 2003; (2) FOOTPRINT, 2006; (3) Verro *et al.*, 2009a; (4) Calliera *et al.*, 2008; (5) Garau *et al.*, 2002; (6) EC, 2002b, (7) EC, 2006

During the productive season 2008 10 fungicides (7 organic and 3 inorganic), 2 herbicides and 2 insecticides (the neonicotinoid thiamethoxam and the organophosphate chlorpyrifos) have been applied. In the first months of the productive season 2009 some of the previous mentioned plant protection products were used: 3 organic fungicides (cyprodinil, fludioxonil and folpet), 2 inorganic fungicides (copper oxychloride and sulfur), one herbicide (glyphosate) and one insecticide (thiamethoxam). In addition, an other fungicide was applied, thiophanate-methyl, whose principal metabolite is carbendazim (estimated maximum occurrence fraction: 0.76; FOOTPRINT, 2006)

3.2.3. Exposure assessment procedures

To assess the exposure, the entire period was divided into three parts: productive season 2008 (from April to September 2008), from October 2008 to the first half of April 2009 and the beginning of the productive season 2009 (from mid April to June 2009).

- PEC estimation for productive season 2008

Within the field, for each a.i. crop interception percentages were subtracted from the application rates. FOCUS (FOCUS, 2001) suggests crop interception values as high as 85% for late growth stage plants and 70% for early growth stage plants in vineyards. Further studies (Ade *et al.*, 2005) give lower values of the fraction of pesticide intercepted by the crop with a traditional application, as in our case: nearly 60% and 40% for tall and small plants respectively. Furthermore, analytical data on the same vineyard after application of chlorpyrifos (Barmaz, 2009) show that FOCUS percentages are too conservative. Joining the information from Ade *et al.* (2005) and Barmaz (2009) a medium value of 50% was used in our calculations.

Additional input to soil may derive from foliar wash-off by rainfall. Every relevant wash-off event has been considered as an application event inside the rows, for insecticides and fungicides, that are sprayed on the plants. Wash-off from leaves has been calculated according to Leistra (2005), as the process to which pesticides undergo after penetration into foliage, photodegradation and volatilisation, when a rain event occurs. Where data were not available, e.g. for penetration into foliage, photodegradation or volatilisation, worst cases of no loss from leaves before wash-off were assumed.

Soil exposure outside the vineyard was assessed by estimating the percentages of added pesticide reaching the soil by drift according to Ganzelmeier *et al.* (1995) modified by Barmaz (2009). For insecticides and fungicides, directly sprayed on the plants, drift amount is 3.6 % and 1.6 % of the application rate, for 4 m 10 m respectively. For herbicides, that are

applied directly at the ground level near the roots, drift was considered as negligible.
Movement of chemicals along the vertical direction in soil is low and not immediate, mainly due to leaching processes after rain, thus the exposure for an organism is due to its movements. Diluting PECs in a different layer of soil may account for these differences. PECs were estimated for a depth of 20 cm, that can be considered as the mean depth in which earthworms, the reference organisms for first tier soil risk assessment, usually live and that is indicated for earthworms sampling (ISO, 2005). PEC value was converted from a mass/surface value into g/kg soil using the soil density measured in lab from samples collected inside the vineyard (0.76 g/cm^3, based on five replicates).
Degradation curves of PECs in soil were calculated according to half life data taken from the literature. To calculate any possible loss from the soil by runoff after rain events the SoilFug model (version 1.2, Di Guardo *et al.*, 1994) has been applied using the following parameters: fraction of organic carbon 0.027 (measured in lab from samples) soil depth 0.3 m, diffusive layer 0.15 m, water fraction 0.3, air fraction 0.2 (Finizio *et al.*, 2005). Rainfall data were collected from San Biagio di Callalta (TV) station (Table 3.1) and outflow data were calculated according to Lutz (1984) and Maniak (1982). The result of the application of the model was a negligible loss.
First order degradation curves in soil after application were constructed from the first application date (28th of April) until the 30th of September 2008, using soil DT_{50}s.

- PEC estimation from October 2008 to mid April 2009

When applications and inputs from wash-off stop, degradation processes into soil and leaching become more important. Leaching was calculated for having an indication whether a substance remains in the soil layer of interest or is transported in deeper layers.
Leaching calculation was performed for organic chemicals excluding glyphosate, which binds strongly to soil particles, and mancozeb, whose DT_{50} in soil is very low (0.5 days). To calculate the depth that a chemical reaches for leaching an equation modified from Bolt and Bruggenwert (1976) was used (eq. 3.1):

$$Xp = \frac{V}{(\varepsilon_l + f_b \cdot Kp)} \qquad \text{(Eq. 3.1)}$$

where:
Xp (cm) is the depth reached by the chemical;
V (cm) is the rain fallen;
ε_l (cm^3/cm^3) is the soil porosity (0.3 used as default value, Finizio *et al.*, 2005);

f_b (g/cm^3) is soil density (0.76, measured in lab from samples);
Kp (L/kg) is partitioning coefficient in soil, calculated as $Kp \approx K_{OC} \times f_{oc}$ (f_{oc} organic carbon fraction in soil). K_{OC} is calculated as $0.41 \times K_{OW}$. (Karickhoff, 1981).
For each rain event from October 2008 and the possible next application in the productive season 2009 *Xp* was calculated according to equation 3.1.
If $Xp > 20$ cm, the chemical moved deeper than the organisms resident layer due to leaching, thus PEC was assumed as negligible.
For $Xp \approx 20$ cm, it was assumed that transport pattern along the soil layer is shaped with the average centered at *Xp* (Bolt and Bruggenwert, 1976), thus it was assumed that half of the concentration of the chemical is above the trigger depth, in the resident layer of organisms. PEC was calculated as 50% of PEC calculated with only degradation curves.
For $Xp < 20$ cm the chemical is assumed to be in the resident layer of earthworms, thus PEC was calculated with first order degradation kinetic equations, without any modification.
For the three inorganic fungicides degradation kinetic equations were taken into account.

- PEC estimation for the beginning of the productive season 2009

On the 15th of April 2009 the applications for the new productive season began. PEC calculations were made following the same procedures described for the productive season 2008. For some chemicals the new load in soil was summed with the residue from 2008.

For characterising long term exposure, time weighted averages (TWAs) instead of the PECs were used. TWAs have been calculated according to equation 3.2:

$$TWA = \int_{t_0}^{t} C_0 e^{-kt} dt = \frac{\left(C_0 - C_0 e^{-kt}\right)}{kt} = \frac{\left(C_0 - C_t\right)}{kt} \qquad \text{(Eq. 3.2)}$$

where:
C_0 is the initial concentration of the chemical, at t_0;
C_t is the concentration of the chemical after a given time t;
t_0 is the initial time;
t is the time when the calculation is stopped;
k is the constant of the first order kinetic degradation curve and is calculated as $ln2/DT_{50}$.

TWA is the most suitable tool to assess long term exposure. Anyway it does not take into account peaks of contamination of substances with a short

DT_{50}. TWA were calculated for 14 days, the time used for long term ecotoxicological test on earthworms, to which exposure has to be compared.

3.2.4. Effect characterization

For risk characterisation, the exposure indicator PEC has to be compared with an effect indicator. For soil risk assessment the organism traditionally used is earthworm, thus the effect indicator is the acute LC_{50} 14 days after the exposure for *Eisenia foetida*. Data for earthworms and when available for microarthropods (the mesostigmatid mite *Typhlodromus pyri* and the springtail *Folsomia candida*) are reported in table 3.4.

Table 3.4. Acute toxicity for soil organisms, the earthworm *Eisenia foetida*, the mite *Typhlodromus pyri* (Acari: Mesostigmata) and the springtail *Folsomia candida* (Collembola: Isotomidae). For *T. pyri* data the values were converted into LC_{50} (mg/kg) from LR_{50} (Lethal Rate 50, g/ha), using the same soil parameters (depth and density) used in the exposure assessment. F: fungicide, H: herbicide, I: insecticide; -: data not available; in italics: a.i. used only on Pinot Grigio variety.

Action	Active Ingredient	Acute LC_{50} (14 days *Eisenia foetida*) (mg/kg)	LC_{50} *Typhlodromus pyri* (mg/kg)*	LC_{50} (35 days) *Folsomia candida*(mg/kg)
F	mancozeb	299.1[(2)]	0.32[(2)#]	-
F	sulfur	1600[(1)]	6.8[(2)]	-
F	dimethomorph	99.5[(2)]	1.18[(2)]	-
H	glyphosate	480[(2)]	-	-
H	oxadiazon	55[(2)]	0.1[(2)]	-
F	folpet	1000[(2)]	-	-
F	iprovalicarb	1000[(1)]	-	-
F	*cyprodinil*	192[(1)]	1.48[(2)]	-
F	*fludioxonil*	1000[(1)]	-	-
I	thiamethoxam	1000[(1)]	-	-
F	copper oxychloride	489.6[(2)]	0.01[(2)]	-
I	chlorpyrifos	210[(1)]	1.3[(2)]	0.2[(2)]
F	copper sulphate	155[(2)]	5.26E-05[(2)]	-
F	*mepanipyrim*	1000[(1)]	-	-
F	*thiophanate-methyl*	13.2[(2)]	0.26[(2) #]	-
F	*carbendazim*	5.4[(1)]	0.66[(2) #]	-

(1) Tomlin, 2003; (2) FOOTPRINT, 2006; * LC_{50} (mg/kg) value derived from LR_{50} (g/ha) value; [#] unverified LR_{50} input data (FOOTPRINT, 2006).

3.2.5. Predicting risk for mixtures

The characterization of risk for each chemical was performed by calculating Exposure/Toxicity Ratios (ETRs) as the ratio between the environmental concentration and a toxicological endpoint. As reported in section 3.2.3, ETRs, were calculated by using the 14 days time weighted averages (TWAs) instead of the PECs.
An example of risk assessment for pesticide mixtures is given, that considers toxicity immediately after an application of chlorpyrifos, one of the most toxic active ingredients used. Moreover, in this 14 days period also other active ingredients are present with high PECs. The TWAs for all chemicals were calculated from individual PECs for the 14 days period following chlorpyrifos application. These TWAs were used for the calculation of ETRs, corresponding to TUs of the mixture.
For predicting risk for the whole mixture, the Concentration Addiction (CA) model by Löewe was used (Greco *et al.*, 1992). This model is applicable to chemicals with the same mode of action, but is commonly used as a reasonable worst case. Indeed, the model suitable for chemical with different modes of action, Independent Action (IA) model by Bliss, requires a detailed information on the modes of action of all the substances involved in a mixture (Greco *et al.*, 1992). In literature, CA model is used as a reasonable worst case, giving higher values than IA applied on the same mixture (Drescher and Boedeker, 1995; Finizio *et al.*, 2005; Junghans *et al.*, 2006). Anyway, it has been demonstrated that the ratio CA/IA even for complex mixture is, in general, relatively low, rarely above one order of magnitude (Drescher and Boedeker, 1995; Finizio *et al.*, 2005), especially considering the fact that 80% of the mixture is generally dominated by a few chemicals (Finizio *et al.*, 2005; Verro *et al.*, 2009b, Chapter II of this thesis).
CA model follows equation 3.3:

$$TUm = \sum_{i=1}^{n} TUi = \sum_{i=1}^{n} \frac{C_i}{EC_{x,i}} \qquad \text{(Eq. 3.3)}$$

where:
C_i is the actual concentration of the individual chemical *i* in the mixture;
$EC_{x,i}$ is the ecotoxicological end-point (e.g. LC_{50}) of the individual chemical *i*;
TU_i are the toxic units of the individual chemical *i*, i.e. the fraction of the ecotoxicological end-point produced by the individual chemical *i* ($TU_i = C_i/EC_{x,i}$);
TU_m are the toxic units of the mixture.

3.3. Results and discussion

3.3.1. Risk assessment

PECs were estimated using the procedures described in section 3.2.3 and the results are shown in figures 3.3, 3.4 and 3.5, divided in inorganic and organic chemicals for sake of clarity of the figures.
PEC values decreased an order of magnitude from A point inside the vineyard to B point, 4 meters away from the last plant row. C point values (10 m away from the last row) are almost half of the PECs in B.
In A point the highest PEC value is reached by sulfur at the end of August, due to the repeated applications during all the productive season. Anyway, its DT_{50} makes it disappear at the beginning of the next productive season. It is not the case of copper (both oxychloride and sulphate): the result of the repeated applications and its very low degradation in soil lead it to persist for the whole period. Other active ingredients with considerable PEC are mancozeb and folpet, but, even if repeatedly applied, they disappear in few days, due to their low DT_{50}s. Also glyphosate is applied three times in 2008: it is assumed to persist, although at PECs as low as 1/8 of the value in September, until the beginning of the following productive season, when its concentration increases due to the following applications. Chlorpyrifos can persist for nearly two month after the end of the productive season and then its concentration in soil decreases due to leaching until it can be assumed as negligible. Oxadiazon shows a similar behaviour, but with higher initial PECs and a lower persistence in soil. Dimethomorph is applied three times, but it is considered to last in soil only a month after the end of the productive season. Iprovalicarb, cyprodinil, fludioxonil and mepanipyrim have low initial PECs compared to the others, they can persist in soil after the productive season 2008 has finished, but just for a month. Thiamethoxam has low initial PECs value and is transported by leaching during the first rainfall events, because of its high water solubility. There is an a.i. applied only in 2009, thiophanate-methyl, that gives as metabolite carbendazim. For them PECs in 2009 were considered only and the result gives low PECs values.
PECs trends in B and C point are similar to those described for A point, just with differences in applied products (herbicides are excluded and on Prosecco plants, close to C point, cyprodinil, fludioxonil, mepanipyrim and thiophanate-methyl were not applied) and, as reported before, the value scale.

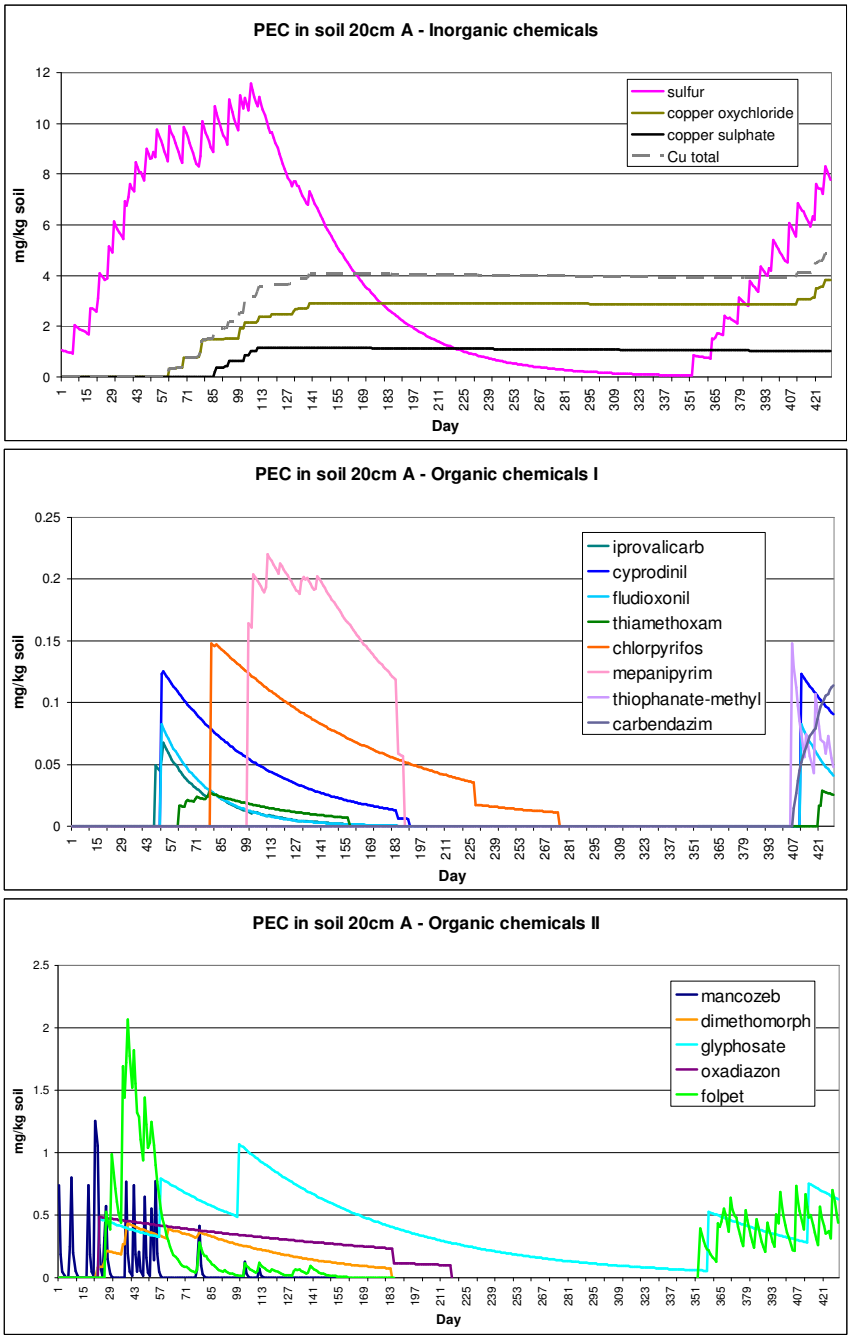

Figure 3.3. PEC (mg/kg soil) in soil (depth 20cm) within the vineyard, under Pinot Grigio variety (divided in inorganic and organic chemicals for sake of clarity).

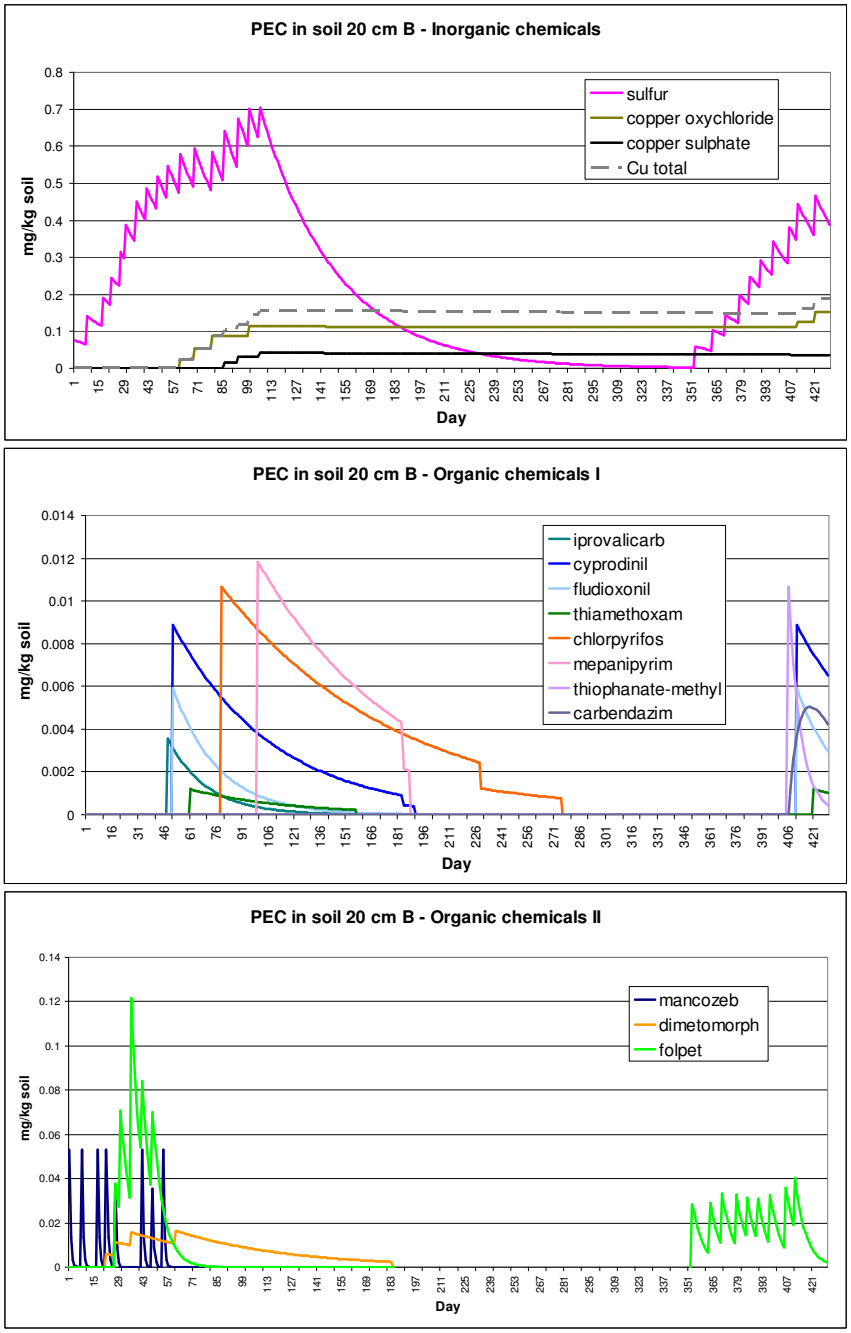

Figure 3.4. PEC (mg/kg soil) in soil (depth 20cm) 4 m away from last row of Pinot Grigio variety (divided in inorganic and organic chemicals for sake of clarity).

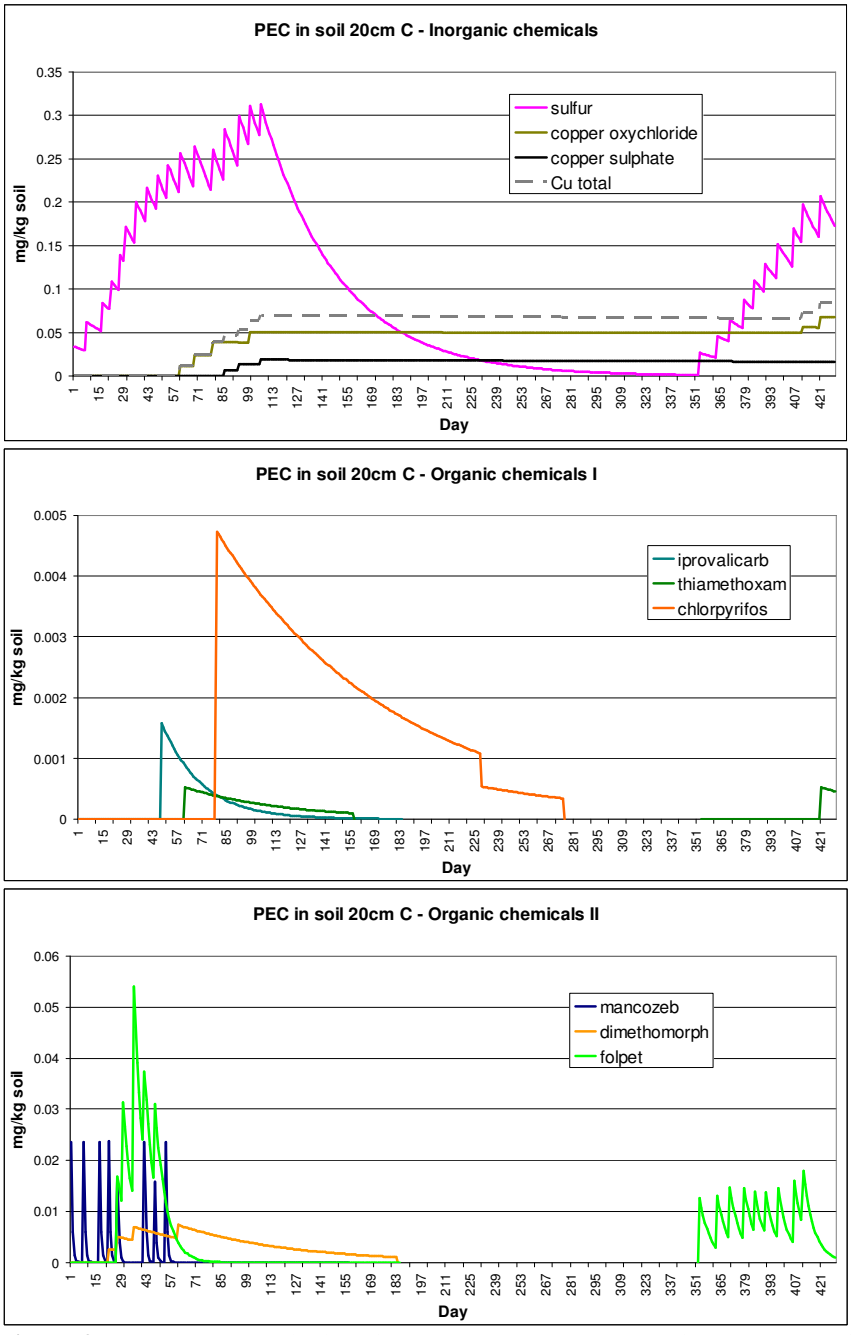

Figure 3.5. PEC (mg/kg soil) in soil (depth 20cm) 10 m away from last row of Prosecco variety (divided in inorganic and organic chemicals for sake of clarity).

TWAs were calculated, according to the procedures described in section 3.2.3 and 3.2.5, for the 14 days period following chlorpyrifos application. Repeated applications and possible additional inputs by rainfall were taken into account. ETRs, or TUs, were calculated from the ratio TWA/LC_{50}. During these 14 days not only chlorpyrifos but also other active ingredients show high PECs, thus it is one of the most contaminated periods in this work.
The risk posed by the mixture is, thus, simply assessed as the sum of the ETRs of individual compounds (table 3.5). To have a time trend of mixture potency, the same approach could be applied to other representative periods of time during the productive season.

Table 3.5. ETRs (equivalent to TUs) 14 days after chlorpyrifos application. F: fungicide, H: herbicide, I: insecticide.

Action	Active Ingredient	ETRs (or TUs) rows (A)	ETRs (or TUs) 4 m (B)	ETRs (or TUs) 10 m (C)
F	mancozeb	1.77E-05	8.22E-21	3.65E-21
F	sulfur	5.96E-03	3.54E-04	1.57E-04
F	dimethomorph	3.28E-03	1.13E-04	5.01E-05
H	glyphosate	1.18E-03	not drifted	not drifted
H	oxadiazon	6.63E-03	not drifted	not drifted
F	folpet	1.22E-04	1.88E-07	8.34E-08
F	iprovalicarb	2.18E-05	6.60E-07	2.93E-07
F	cyprodinil	3.63E-04	2.53E-05	not applied
F	fludioxonil	2.17E-05	1.56E-06	not applied
I	thiamethoxam	2.32E-05	7.61E-07	3.38E-07
F	copper oxychloride	3.02E-03	1.78E-04	7.89E-05
I	chlorpyrifos	6.74E-04	4.74E-05	2.11E-05
F	copper sulfate	1.64E-03	4.96E-05	2.20E-05
F	mepanipyrim	not applied	not applied	not applied
F	thiophanate-metyl	not applied	not applied	not applied
F	carbendazim	not present	not present	not present
	MIXTURE	2.30E-02	7.69E-04	3.30E-04

From the estimate of the effect of the mixture, the contribution of individual chemicals to joint toxicity can be estimated (figure 3.6).

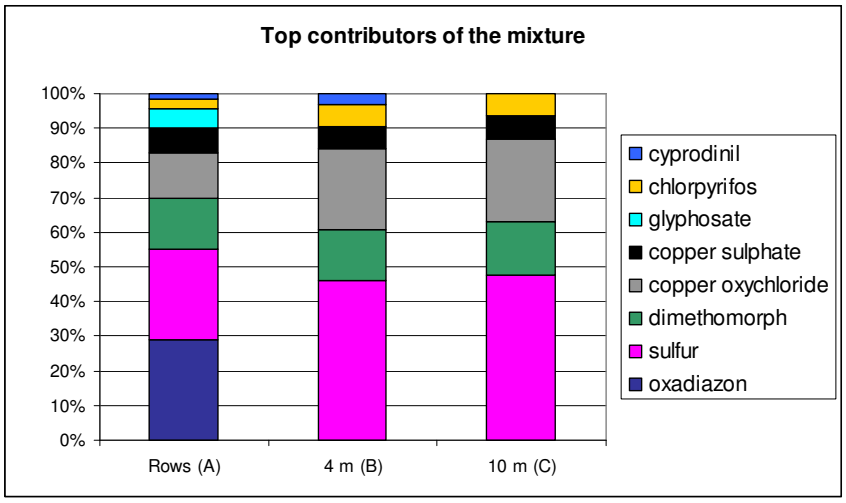

Figure 3.6. Percentage contribution of the active ingredients in the overall mixture toxicity.

This analysis reveals that the mixture is highly influenced by a few compounds: notably oxadiazon, sulfur, dimethomorph and copper oxychloride. The two inorganic compounds, especially sulfur, dominate the total amount of pesticide added as a result of repeated applications, although their toxicity is low. In contrast, oxadiazon and dimethomorph contribute a lesser amount to the predicted risk even though they have greater toxicity, while chlorpyrifos which is one of the most toxic compounds for earthworms provides only the seventh largest contribution to total TUs due to the comparatively low PECs. Overall, risk for mixture in the soil within the field is relatively low but, considering that TUs are calculated on the basis of acute LC_{50}s, a threat of the pesticide mixture for the in field soil community cannot be excluded. For sampling points outside the field, risk is more than three orders of magnitude lower than one acute TU, thus it can be assumed as negligible.

Risk assessment based on earthworms toxicity data underestimates the results for the whole soil community, especially for microarthropods, that are usually the target organisms of insecticides. Indeed, springtails are three order of magnitude more sensitive to chlorpyrifos than earthworms, while mites are two order of magnitude more sensitive (see table 3.4). Also for fungicides and herbicides mites are more sensitive: one order of magnitude for carbendazim, two for dimethomorph, oxadiazon, cyprodinil and thiophanate-methyl, three for mancozeb and sulfur, four for copper after copper oxychloride application and even seven after a copper sulphate application. These data are shown only to give an idea of the differences, because were extrapolated with different methods (an ecotoxicological test

for earthworm and a calculation for mite). The information that are given for microarthropods are usually referred to application rates and multiples (half and doubled), in a completely different way as for earthworms. There is thus a need of more specific effect data on microarthropods, to perform a more complete risk assessment; only a few data are available in ecotoxicological databases, for just a few chemicals.

For risk validation some ecotoxicological tests were made by NO MIRACLE partners, using the soil sampled in field as medium for tests with *Eisenia foetida*. Soil was sampled in the three stations (A, B and C) in pre-application of insecticides (20th May 2008), immediately after an application of chlorpyrifos (15th July 2008, the same day of the application) and in September, (16th September 2008). They sieved the soil samples, dried them and used them to prepare 5 replicates for each point and date. Five blank replicates were prepared using Kettering soil with 3% of composted bark at 30% moisture on dry weight. 8 earthworms were added to each jar, wet manure was used as food source and water was added to sample replicates to adjust soil moisture. Survival, weight loss and manure consumption were measured after 2 and 4 weeks, while cocoon production after 4 weeks, but results did not show significant effects (Spurgeon, unpublished data). This is not surprising considering the results of the theoretical risk assessment.

3.3.2. Risk mapping

Risk assessment has been performed at field scale, following the procedures described in section 3.2. For mapping risk in a wider area, thus upscaling the risk assessment, some considerations are needed.
As shown in risk assessment results (section 3.3.1), risk for organisms dramatically decrease outside the considered field. For this reason, for risk mapping on a large scale only fields are taken into account, considering negligible the risk outside them.
Site specific risk assessment procedure can be upscaled to the large scale considering as surface at risk the total area on which a substance is used, i.e. the sum of the surfaces of the fields on which a particular product is applied. Additional information are needed:

- the application rate of the chemical (kg/ha),
- the percentage of the area on which the chemical is used (ha) or the total amount applied in the area (kg).

The geographical unit of risk is the field. For risk mapping, the area can be divided into smaller areas (e.g. with a grid) and the number of fields with the same level of risk due to the application of a particular a.i. should be estimated for those areas. The risk level of each cell of the grid is due to the percentage of fields with a fixed risk level.

Figure 3.7 shows an example of risk mapping applied on a Spanish site for fenitrothion. The unit of the map is a 1 × 1 km square. The most suitable map unit would be a function of the detail of land use and pesticide use information.

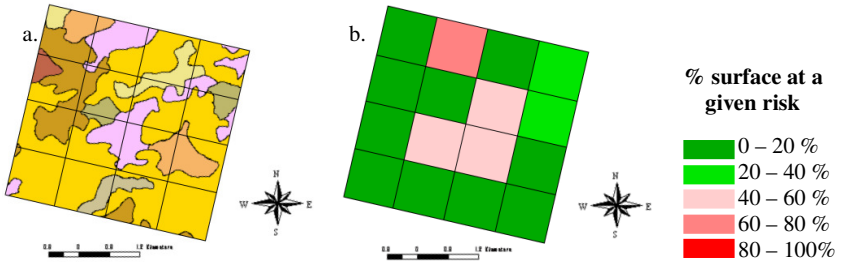

Figure 3.7. CORINE (EEA, 2000) land use in an agricultural area (4 × 4 square) in San Pere de Ribes, Spain (vineyards in purple) (a) and risk mapping for a given a.i., as a function of land use, at a given risk (b). (modified after Vaj and Vighi, 2009).

Moving to the regional scale, getting reliable information on pesticide use is difficult. Estimates can be done as a function of land use (e.g. CORINE land cover data; EEA, 2000) and usual agricultural practices.

3.4. Conclusions

The risk assessed with the first tier traditional methods for one of the most contaminated periods in the site-specific work is relatively low, even within the field. Tests performed on earthworms by NO MIRACLE partners confirm that these organisms are not affected by the actual plant protection product applications. Risk outside the treated field is considered as negligible.

Nevertheless, calculations and comparisons among toxicity on different soil species were made. Even it is a first rough step and deeper research in this direction are needed, they indicates that microarthropods are orders of magnitude more sensitive to pesticides than earthworms, not only regarding insecticides, that could seem obvious, but also for fungicides and herbicides. It seems thus more appropriate not to consider them as organisms to test in higher tier investigations, but the actual community on which perform the risk assessment in the soil compartment. For this purpose standardised methods to calculate LC_{50}s are needed, for providing the right endpoint value for a first tier risk assessment on this community.

Investigations on natural microarthropods community in the same field have been performed using the exposure assessment performed here as basis and are reported in chapter V.

Acknowledgments

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References

Ade G, Molari G, Rondelli V, 2005. Vineyard evaluation of a recycling tunnel sprayer. *T asae* 48: 2105-2112.

Bakker FM, Feije R, Grove AJ, Hoogendoorn G, Jacobs G, Loose ED, van Stratum P, 2002. A laboratory test protocol to evaluate effects of plant protection products on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) in standard soil. *J Soils Sediments* 3: 73-77

Barmaz S, 2009. *Plant protection product risk assessment: distribution and experimental validation in terrestrial ecosystems.* PhD thesis, Doctorate in Environmental Sciences (XXII cycle), Faculty of Mathematical, Physical and Natural Sciences, University of Milano – Bicocca, Milano, Italy, 113 pp. http://hdl.handle.net/10281/7503.

Bolt GH and Bruggenwert MGM (Eds.), 1976. *Soil chemistry, A: Basic elements*. Developments in Soil Science 5A. Elsevier, Amsterdam, The Netherlands, 281 pp.

Calliera M, Balderacchi M, Capri E, Trevisan M, 2008. Prediction of agrochemical residue data on fruit using an informating system (PARDIS model). *Pest Manag Sci* 64: 981-988.

Collemback JH, 1982. Loss of herbicides from ground sprayers. *Weed Res* 22: 193-204.

Di Guardo A, Calamari D, Zanin G, Consalter A, Mackay D, 1994. A fugacity model of pesticide runoff to surface water: development and validation. *Chemosphere* 28: 511-531.

Drescher K and Boedeker W, 1995. Assessment of the combined effects of substances: the relationship between concentration addition and independent action. *Biometrics* 51: 716–730.

EC (European Commission), 1991. Council Directive 91/414/EEC concerning the placing of plant protection products on the market. *Off J Eur Communities* L 1991; 230 19/8/1991.

EC (European Commission), 2002a. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002 rev 2 final, 17 October 2002.

EC (European Commission), 2002b. Review report for the active substance iprovalicarb, SANCO/2034/2000-FINAL, 2 July 2002.

EC (European Commission), 2003. *Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC for the new notified substances and the Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC on biocides*. Second ed. European Commission, Louxembourg, Part 1, 2 and 3, 760 pp.

EC (European Commission), 2006. Review report for the active substance thiamethoxam, SANCO/10390/2002 - rev. final, 14 July 2006.

EEA (European Environmental Agency) website, 2000. Corine Land Cover - data download for year 2000. http://www.eea.europa.eu/themes/landuse/clc-download (accessed in July 2009).

Finizio A, Villa S, Vighi M, 2005. Predicting pesticide mixture load in surface waters for a given crop. *Agr Ecosyst Environ* 111, 111-118.

FOCUS, 2001. *FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC*. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.

FOOTPRINT website, 2006. The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.eu-footprint.org/ppdb.html (accessed in June 2009).

Ganzelmeier H, Rautmann D, Spangenberg R, Streloke M, Hermann M, Wenzelburger HJ, Walter HF, 1995. *Studies on the spray drift of plant protection products. "Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft"*. No 305, Blackwell Wissenschafts-Verlag, Berlin, Germany, 111 pp.

Garau VL, Angioni A, Aguilera del Real A, Russo M, Cabras P, 2002. Disappearance of azoxystrobin, pyrimethanil, cyprodinil, and fludioxonil on tomatoes in a greenhouse. *J Agric Food Chem* 50: 1929-1932.

Greco W, Unkelbach HD, Pöch G, Suhnel J, Kundi M, Bodeker W, 1992. Consensus on concepts and terminology for combined-action assessment: the Saariselkä agreement. *Arch Complex Environ Stud* 4: 65-72.

Hilbert G, 1992. *Litteraturstudier om pesticiders afdrift, afsætning pa kantbiotoper og fjerntranport.* Arbejdsrapport fra Miljøstyrelsen 59: 1-23. In Danish.

ISO (International Organization for Standardization), 1999. ISO 11267 *Soil quality. Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants.* International Organization for Standardization, Geneva, Switzerland, 12 pp.

ISO (International Organization for Standardization), 2006. ISO/FDIS 23611-1 *Soil quality- Sampling of soil invertebrates – Part 1: Hand sorting and formalin extraction of earthworms.* International Organization for Standardization, Geneva, Switzerland, 16 pp.

Junghans M, Backhaus T, Faust M, Scholze M, Grimme LH, 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquat Toxicol* 76: 93-110.
Karickhoff SW, 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* 10: 833-846.
Koch H and Weisser P, 2001. Spray deposits of crop protection products on plants – the potential exposure of non-target arthropods. *Chemosphere* 44: 307-312.
Leistra M, 2005. *Estimating input data for computations on the volatilisation of pesticides from plant canopy and competing proceses*. Alterra-rapport 1256, Alterra, Wageningen, The Netherlands, 80 pp.
Løkke H and Van Gestel CAM, 1998. *Handbook of soil invertebrate toxicity tests.* Ecological and Environmental Toxicology Series, Wiley and Sons, Chichester, UK, 281 pp.
Lutz W, 1984. *Berechnung von hochwasserabflussen unter anwendung von gebietskenngrossen.* Mittlg. Inst. Hydrologie Wasserwirtschaft, Univ. Karlsruhe, Heft 24. In German.
Maniak U, 1992. Regionalisierung von parametern fur hochwasserabflussganglinier. In: HB Kleeberg (ed.), *Regionalisierung der hydrologie*, DFG, Mittlg. Senatskormm. Fur Wasserf. 11: 325-332. In German.
Rautmann D, Streloke M, Winkler R, 2001. New basic drift values in the authorization procedure for plant protection products. In: Forster R, Streloke M, *Workshop on risk assessment and risk mitigation measures in context of the authorization of plant protection products (WORMM)*. Mitt. Biol. Land – Forstwirtsch. No. 381. Berlin – Dahlem, Germany.
Tomlin CDS (Ed), 2003. *The pesticide manual: a world compendium (13th ed.)*. British Crop Protection Council, Farnham, UK, 1606 pp.
Vaj C and Vighi M, 2009. Assessing and mapping pesticide risk on ecosystems. III: soil community. Presented to: NO MIRACLE final conference "Multiple Stressors – Novel Methods for Integrated Risk Assessment", 28th – 30th September 2009, Århus, Denmark.
Vaj C, Van Gestel CAM, Vighi M, 2010. Year-round monitoring of soil microarthropod communities under pesticide application and other stressors in a real case. Presented to SETAC Europe XX meeting "Science and technology for environmental protection", 23rd – 27th May 2010, Seville, Spain.
Verro R, Finizio A, Otto S, Vighi M, 2009a. Predicting pesticide environmental risk in intensive agricultural areas. I: screening level risk assessment of individual chemicals in surface waters. *Environ Sci Technol* 43: 522-529.
Verro R, Finizio A, Otto S, Vighi M, 2009b. Predicting pesticide environmental risk in intensive agricultural areas. II: screening level risk

assessment of complex mixtures in surface waters. *Environ Sci Technol* 43: 530-537.
Vicari A, Otto G, Zanin G, 2001. Dinamica ambientale degli erbicidi, In: Zanin G and Catione P (Eds.), *Malerbologia*. Pàtron Editore, Bologna, Italy, pp. 523-580. In Italian.
Wang M, Rautmann D, 2008. A simple probabilistic estimation of spray drift – factors determining spray drift and development of a model. *Environ Toxicol Chem* 27:2617-2626.

CHAPTER IV

Consideration on differences in risk assessment among different compartments

Abstract
Mixture toxicity is a real world problem and as such requires risk assessment solutions that can be applied within different geographic regions, across different spatial scales and in situations where the quantity of data available for the assessment varies from very rich to relatively poor. Moreover, the need for site specific procedures for assessing ecotoxicological risk for non target species in receiving ecosystems also has to be recognised.
The considerations given in this chapter addresses the actual effects of pesticide mixtures on natural communities in three different compartments: freshwater, soil and terrestrial aboveground. It refers to chapter II, III and to a risk assessment procedure for pollinators developed by Barmaz *et al.* (2010) within the ALARM project. A field validation of the exposure index proposed was conducted in collaboration with Dr. Stefania Barmaz and gave satisfactory results.
The three work discussed refer to the same study area, River Meolo basin (Northern Italy), a catchment characterised by intensive agriculture. Value and limitation of the approaches are described, discussing differences in the three compartments. The possibilities for larger scale applications in risk assessment are also discussed.

Keywords: aquatic, soil, pollinators, GIS.

Parts of this chapter are contained in: Vaj C, Barmaz S, Søresen PB, Spurgeon D, Vighi M. Assessing, mapping and validating site-specific ecotoxicological risk for pesticide mixtures: a case study for small scale hot spots in aquatic and terrestrial environment. *In preparation.*

4.1. Introduction

The procedures currently used for assessing ecotoxicological risk for environmental chemicals are usually based on standard approaches, suitable to be applied in a transparent way, in order to fulfil the regulatory requirements for toxic substances. The risk characterisation process requires a comparison between exposure values, such as a PEC (Predicted Environmental Concentration), and effect parameters, such as a PNEC (Predicted No Effect Concentration). The PEC is generally estimated via the application of predictive models, while the PNEC values are usually derived from laboratory toxicity data, often generated using the standard test procedures.

An exception to the most commonly used traditional risk assessment approach is the procedure that is used for pollinators. In Europe, the production of the 84% of crop species depends directly on insect pollinators (Williams, 1994). In agro-ecosystems these key species are potentially at risk because of insecticides use. To assess this risk, the most used procedures are based on approaches, such as the Hazard Quotient (HQ) calculated as the ratio between pesticide application rate (g/ha) and a toxicological endpoint (μg/bee). Unless ETR (Effect/Toxicity Ratio) approaches used in other risk assessment procedures, HQ doesn't have any quantitative meaning of exposure evaluation.

The standard procedures developed for regulatory risk assessment purposes represent a powerful tool for the classification of potentially hazardous chemicals and serve as tool to quantify the relative risk. However, the results are by no means truly representative of actual site-specific conditions and so are difficult to relate to the risk posed to real ecosystems. Laboratory toxicity tests that represent the starting point for conventional risk assessment procedures cannot, of course, account for the true complexity of community level responses to chemical exposure. For one thing, current risk assessment procedures are mainly focused on the effect of individual chemicals and not on the effects of environmentally relevant mixtures as would be expected in many receiving ecosystems. For true effect prediction, this is a considerable weakness.

As a step towards risk estimation for catchments and individual sites, spatial explicit models built within geographical information systems (GIS), can provide a means to convert information on source distribution, land use information and catchment hydrology to a local and regional scale exposure assessment (Sumpter *et al.*, 2006). Derivation of catchment risk, through comparison of derived PECs with PNEC derived from laboratory toxicity data, can then provide information on the distribution of potential risks in space and time as represented by a risk ratio.

In this work, a case study that outlines site specific approaches to assess effects of multiple stress factors on ecosystems utilising exposure modelling

and risk assessment is presented. The approaches developed are initially applied to the small-scale catchments of Meolo and upper Livenza river basins, Northern Italy, although their general applicability is discussed. The focus is on the risk assessment of pesticides, which are assumed to be the class of chemicals that are most relevant for assessing environmental risk. In the same experimental areas, site-specific pesticide risk assessment has been performed on the aquatic environment (chapter II), on the terrestrial hypogean (soil community, chapter III) and on the terrestrial epigean (pollinator community) biota. The objectives of the chapter is to give a general overview of different risk assessment approaches applicable to different environmental compartments, but in the same geographic area, in order to compare the level of damage from multiple stress factors on different communities, under the same anthropogenic pressure. The possibility of extending the spatial risk assessment approach for use in hot-spot identification at the regional, national and continental scales is also discussed.

Risk assessment on aquatic and terrestrial belowground compartments have been described in the previous chapters. In this chapter, a procedure for assessing risk for pollinators, developed within the ALARM (Assessing LArge scale environmental Risks for biodiversity with tested Methods) project of the European Commission by Dr. Stefania Barmaz and an experimental validation activity are reported.

4.2. Risk assessment for pollinators

In the absence of a method for assessing pesticide exposure to pollinators, the HQ method is regularly applied. This is, however, a simplistic approach and more holistic methods are needed. Therefore, a new procedure for the site-specific assessment of pesticide exposure and risk for pollinator has been developed by Barmaz (2009) and Barmaz *et al.* (2010). They calculated TDI (Total Daily Intake) for contact and oral exposure as a function of PEC on plants within the home range of bees (assumed as the representative organism for the pollinator community). They calculated PEC within treated fields from application rates and crop interceptions and assessed exposure trend according to Leistra (2005).
To assess exposure on non crop vegetation outside the field, a semi-quantitative exposure index based on the drift equations derived from Ganzelmeier *et al.* (1995) was developed. The index, reported in Barmaz *et al.* (2010), is shown in equation 4.1:

$$FC_s = \frac{\sum_{i=1}^{n}[AR \cdot (y) \cdot d \cdot p_i]}{St \cdot LAI} \quad \text{(Eq. 4.1)}$$

where:
FC_s is the foliage concentration (μg/cm^2);
AR is the application rate (μg/cm^2);
d is the buffer width (10 cm);
p_i is the perimeter of the field (cm);
St is the surface area considered (cm^2);
LAI (cm^2/cm^2) is the Leaf Area Index;
n is the number of fields treated with a given active ingredient.
y is the fraction of substance drifted off, resulting from the following equation (eq. 4.2):

$$y = \left(\frac{1}{1+ax}\right) \cdot b^{-cx} \quad \text{(Eq. 4.2)}$$

where:
x is the distance from the field (m);
a, b and c are the coefficients of the curves derived from Ganzelmeier *et al.* (1995), specific for each crop type and phenological stage.

The exposure assessment procedure for non-crop vegetation outside field was validated during the productive season 2008, in a site in the Meolo river basin (Veneto region, Northern Italy). In the area the main crop on which insecticide are applied, vineyard, covers nearly 400 ha, i.e. nearly ¼ of the basin surface. A 4 × 4 km square was identified in this area and divided into 1 × 1 km smaller squares (site 1). Since the purpose of the index is to give a semi-quantitative estimate of exposure for the large scale, 16 points in 16 km^2 were assumed as suitable. A control site was identified in the same region, but in an agricultural area with a few vineyards and considerably less insecticide impact (site 2). The vineyards present in the areas were mapped using aerial photographs, GIS (ArcView 3.1; ESRI, 1996) and direct observations in field (figures 4.1 and 4.2).

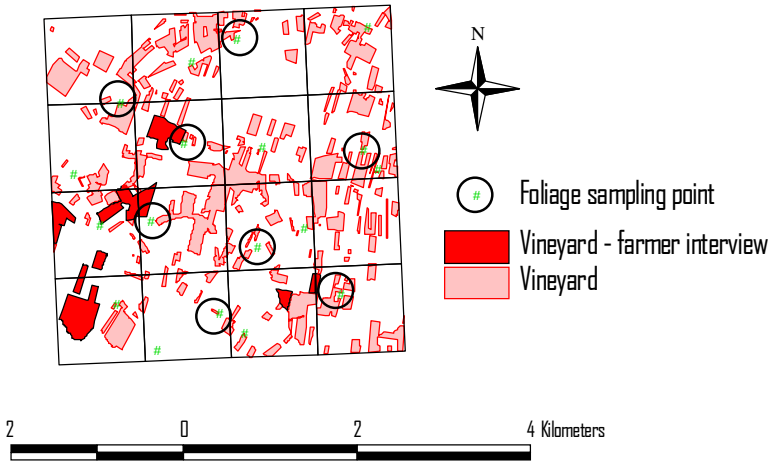

Figure 4.1. 4 × 4 square with high vineyard cultivation (site 1).

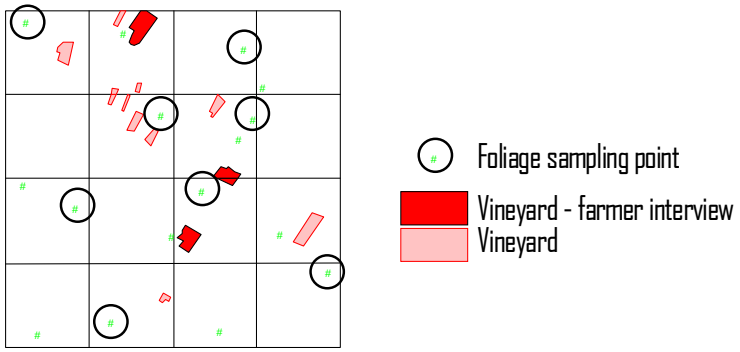

Figure 4.2. 4 × 4 km square with low vineyard cultivation (schematic drawing without right orientation and fictitious scale) (site 2).

Chlorpyrifos application pattern was assessed through farmer interviews, proportions from these data (only for site 2, due to the low extent of vineyard cultivations) and sales data, identifying realistic application dates on expert judgment basis (table 4.1).

Table 4.1. Chlorpyrifos application dates and amounts for the pre-sampling dates in the two sites.

Site	Date	Amount (kg)	Treated area (ha)	Estimated application rate (kg/ha)	Source
1	6-Jun-08	7.5	18.7	0.40	Farmer interviews
	26-Jun-08	3	7.22	0.42	Farmer interviews
	11-Jul-08	9.32	18.7	0.50	Farmer interviews
	11-Jul-08	94.5	152.6	0.62	Sales data
	15-Jul-08	3	7.22	0.42	Farmer interviews
2	16-Jun-08	3.75	7	0.54	Farmer interviews
	16-Jun-08	1.77	3.3	0.54	Proportion from interviews
	1-Jul-08	3.75	7	0.54	Farmer interviews
	1-Jul-08	0.88	3.3	0.27	Proportion from interviews
	2-Jul-08	6.43	10	0.64	Farmer interviews
	2-Jul-08	1.51	4.7	0.32	Proportion from interviews

A non-crop vegetation sample was collected for each small square after the main application date (16th July 2008): nearly 100 g of foliage were taken from a height between 1 and 2 m, packaged in aluminium foil and stored at -20°C prior to the analyses.
Eight samples per site were analysed. Extractions were made in two replicates by ultrasonication (3 cycles of 15 minutes), clean-up was performed with graphitized carbon black (GCB) SPE and samples were analysed with GC-MS. The method gave a LOD of 0.8 ng/g dw and 95% of recovery percentages evaluated for different levels of contamination (RSD=13). For details on this procedure see Barmaz (2009).

Experimental results were compared with concentrations predicted with the index (table 4.2).

Table. 4.2. Measured and predicted chlorpyrifos concentrations on leaves from the two areas. Data for site 1 were calculated by Barmaz (2009).

	High vineyard pressure area (site 1)		**Low vineyard pressure area (site 2)**	
Sample n.	**Measured (µg/g dw.)**	**Predicted (µg/g dw.)**	**Measured (µg/g dw.)**	**Predicted (µg/g dw.)**
1	0.13	0.17	*nd*	0.0009
3	0.08	0.18	*nd*	0
6	0.32	0.40	*nd*	0.002
8	0.30	0.61	0.13	0.0015
9	*nd*	0.18	0.14	0
11	0.26	0.35	0.03	0.0017
14	0.21	0.33	*nd*	0.0004
16	0.25	0.34	*nd*	0
mean	0.22	0.32	0.034	0.0008
sd	0.08	0.15	0.06	0.0008

Measured and predicted results are comparable for site 1, while in site 2, less contaminated by the insecticide, the equation developed underestimates the concentrations. It seems that, being site 2 with low vineyard pressure the foliage contamination is more depending on parameters not taken into account in the index, e.g. wind speed and directions, volatilisation from crops and subsequent deposition. In contrast, in site 1, with high vineyard pressure, these parameters can be less important that those included in the equation 4.1. Anyway, the equation proposed by Barmaz *et al.* (2010) is not a model that gives precises numbers as output, but a semi-quantitative index that should be able to give an indication of contamination. With this consideration, the results seem satisfactory, especially for site 1.

A complete risk assessment procedure for pollinators is proposed and described by Barmaz *et al.* (2010).

4.3 Discussion

All procedures described (chapters II and III and section 4.2) utilise a GIS-based framework (land use, pesticide use, hydrographic network, soil properties, meteorological data, etc). All the results are georeferenced and maps of exposure and risk can be developed as a tool to assist in the visualisation of risk and as a support to decision making. To date the work described has been performed at the small scale, from small basin to the field scale, where detailed information on several environmental issues (land use, hydrology, soil properties, etc.) can be obtained. For a practical usefulness of

GIS-based approaches to be fully established, there remains a need for upscaling the approach with each aspect requiring particular considerations.
The possibilities for GIS approaches for the terrestrial and aquatic environment are notably different because the three ecosystem typologies considered typically require a completely different geographical definition. In particular, the landscape units required are not the same.

1. For the aquatic environment (in particular rivers) the geographic unit is the hydrographic basin. Pesticides reach the water body trough drainage, runoff or drift deposited on the network of ditches. Runoff and drift models allow prediction of pesticide concentration at the outlet of the basin. As reported in chapter II, the predictive capability of these models is evident by the validation undertaken by chemical and biological measurement (Bonzini *et al*., 2006; 2008). Dividing river basins into small sub-basins may allow producing risk maps with high level of detail, although such models are data intensive. Examples of application of the procedure described in chapter II for producing risk maps at the local and regional scale are reported by Verro *et al.* (2002), Sala and Vighi (2008) and Pistocchi *et al.* (2010). Applying the approach at a wider scale (national, continental) is feasible without conceptual difficulties, by applying suitable existing models. Although a practical obstacle in many cases will be the availability of reliable data, in particular on catchment hydrology and pesticide usage (Bonzini *et al.*, 2006).
2. For the soil system, drift is considered the main process in determining an exposure. Rainfall contaminated by pesticides could be a further input, especially for their extent on an area, but the load is considered to be low. As an example, Vighi and Calliera (1999) measured very high concentration values of azinphos-methyl (as high as 1 µg/L) in rainfall in an orchard cultivated area near Forlì (FC, Italy) in 1994. Anyway, these concentrations can be considered as non relevant in soil, if compared to the PECs due to drift calculated a few meters outside a field (e.g. chapter III of this thesis). Therefore, the main the geographic unit is the field and the part of soil involved in direct drift. The limited extent of drift in most cases means that it is unlikely that risk to the soil community will occur at some meters from field boundaries. Consequently, each field represents a fully different exposure situation (differing in crop, active ingredients used, application amounts and dates, etc.). In this case, quantitative maps of exposure and risk can only be made at the local scale. On a wider scale, only semi-quantitative approaches are possible, based on maps on information on crop density and pesticide usage statistics for relevant crops. In these case, risk may be expressed as a function of the surface covered by a given crop and treated with a given pesticide (with known application rate) within a given geographic unit. The geographic unit of the map is a function of the level of detail of the

information available. These kinds of maps have only a comparative value. The actual, site-specific threat for the soil community cannot be quantified. An example of risk mapping and upscaling is given in chapter III and in Vaj and Vighi (2009).

3. For pollinators, the exposed community can move toward and within the exposure areas, thus the geographic unit can be defined as a function of the foraging area. Pesticide concentration within the foraging area may be very variable. However, the TDI of the considered organism can be assumed to be the result of a weighted average within the foraging area and, for large scale mapping, only a semi-quantitative assessment of risk can be obtained. An example is given in Barmaz and Vighi (2009). However, a more precise representation of the actual, rather than only comparative, risk can be obtained. Conceptually comparable approaches, based on a combined information on pesticide use and on the ecological behaviour of the target organisms can be applied to other terrestrial epigean communities, such as birds (Sala *et al.*, 2010).

Besides spatial distribution, the time trend of pesticide risk is also different in the different ecosystems. In rivers (especially small rivers) exposure is characterised by a series of pulses (peaks) depending upon rain events. In terrestrial ecosystems peaks, corresponding to application dates, are followed by a decrease depending upon fate parameters (volatilisation, biodegradation, photodegradation, etc.). Wash-off corresponding to rain events usually may produce a decrease of exposure in vegetation coupled with a moderate increase in soil and increased runoff to surface waters.

For practical purposes (e.g. for regulatory purposes), and in particular if the toxicological endpoint considered is acute (short term toxicity) risk can be characterised on the basis of peak concentrations, assumed as a worst case for conservative assessment, for both aquatic and terrestrial ecosystems. For more precise assessments, and particularly for medium-long term exposure, the time trend should be more carefully accounted. The use of a time weighted average (TWA) could be a reasonable approximation.

For all described examples, the assessment of mixture response is based on the Concentration Addition (CA) approach. CA is an easily implemented approach that is applicable to non-interactive mixtures of similarly acting chemicals that can provide a rough approximation of potential toxicity for particular complex mixtures. For several reasons, already mentioned in chapter II and III, CA represents an acceptable, pragmatically applicable, compromise for practical purposes. The precautionary nature of CA compared to IA means that this model can be applied for regulatory studies even in cases where the conceptual basis for the model are not fully met.

This is confirmed by the composition of the mixtures studied in this thesis, where a few chemicals are responsible for the toxic potency of the mixture. A relevant potential limitation of the CA approach is the impossibility to account for possible synergisms. At the present status of scientific

knowledge, there are no models available capable to predict synergistic (or antagonistic) effects, which must be considered case by case. As a result, such interactions remain as uncertainties within the assessment that may need to be considered.

4.4. Conclusions

The objective of this chapter, linked to II and III, was to give a general overview of an integrated methodological approach for assessing and mapping pesticide risk in different systems, highlighting value and limitations of the procedures as well as their potential for hot spot definition and large scale mapping. As a final step, experimental validation of the reliability of the theoretical approaches and of its suitability for predicting actual effects on natural communities can be undertaken and results compared to model predictions.

Referring to exposure, the theoretical predictive approaches can and have been validated through experimental monitoring (Bonzini *et al.*, 2006; Barmaz, 2009). It is however more complex to validate prediction of risk as this requires studies on structural and functional characteristics of community that take into account the possible interferences with stress factors other than pesticides.

For the aquatic environment, the results obtained through studies on macrozoobenthos demonstrated that a potential risk predicted with theoretical approaches corresponds to substantial changes in the biological community and that these changes depend, at least in part, upon pesticide toxicity (Bonzini *et al.*, 2008).

A series of systematic surveys on biodiversity activity of the pollinator community was developed in the European experimental sites included in ALARM project (Hammen *et al.*, 2009). The methodologies used in the surveys are standardized and an evaluation of their efficiency had been recently done (Westphal *et al.*, 2008). The validation of risk assessment predictions with data on pollinator communities is ongoing.

For the terrestrial ecosystems, experimental studies on the exposed communities were performed and reported in chapter V.

It should be emphasised that uncertainty related to the task of finding relationships between pesticide applications and effect in ecosystems comes from several sources. The models predicting exposure levels are uncertain and this is also the case for the toxicity data that are used to interpret the exposure in terms of potential effects on ecosystems. In particular, a few organisms are used as rough estimates of whole ecosystems and simple laboratory testing is mimicking highly complex effect relations. This means that the estimation of risk is by default uncertain. Uncertainty also applies to the description of the actual conditions in relation to pesticide effects. This

includes observation and interpretation of ecosystem effects both in relation to how the data are collected to represent the communities and in relation to assumed structures of species distributions. As a result, the "true" effect on the ecosystem may easily be masked by variation within the system and thus not be seen as significant. Moreover, co-variations between other factors than pesticides among the agriculture activities and impacts on ecosystems makes it potentially difficult to see a clear signal for pesticides.

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References

Barmaz S, 2009. *Plant protection product risk assessment: distribution and experimental validation in terrestrial ecosystems.* PhD thesis, Doctorate in Environmental Sciences (XXII cycle), Faculty of Mathematical, Physical and Natural Sciences, University of Milano – Bicocca, Milano, Italy, 113 pp. http://hdl.handle.net/10281/7503.

Barmaz S and Vighi M, 2009. Assessing and mapping pesticide risk on ecosystems. I: epygean terrestrial communities – pollinators. Presented to: NO MIRACLE final conference "Multiple Stressors – Novel Methods for Integrated Risk Assessment", 28th – 30th September 2009, Århus, Denmark.

Barmaz S, Potts S, Vighi M, 2010. A novel method for assessing risks to pollinators from plant protection products using honeybees as a model species. *Ecotoxicology* 19: 1347-1359.

Bonzini S, Verro R, Otto S, Lazzaro L, Finizio A, Zanin G, Vighi M, 2006. Experimental validation of a GIS-based procedure for predicting pesticide exposure in surface water. *Environl Sci Technol* 40: 7561-7569.

Bonzini S, Finizio A, Berra E, Forcella M, Parenti P, Vighi M, 2008. Effect of river pollution on the colonisation of artificial substrates by macrozoobenthos. *Aquat Toxicol* 89: 1-10.

ESRI (Environmental Systems Research Institute), 1996. *Using ArcView GIS*. ESRI Inc., Redlands (CA), USA, 340 pp.

Ganzelmeier H, Rautmann D, Spangenberg R, Streloke M, Hermann M, Wenzelburger HJ, Walter HF, 1995. *Studies on the spray drift of plant protection products. "Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft".* No 305, Blackwell Wissenschafts-Verlag, Berlin, Germany, 111 pp.

Hammen VC, Biesmeijer JC, Bommarco R, Budrys E, Christensen TR, Fronzek S, Grabaum R, Jaksic P, Klotz S, Kramarz P, Kröel-Dulay G, Kühn I, Mirtl M, Moora M, Petanidou T, Pino J, Potts SG, Rortais A, Schulze CH, Steffan-Dewenter I, Stout J, Szentgyörgyi H, Vighi M, Vujic A, Westphal C, Wolf T, Zavala G, Zobel M, Settele J, Kunin WE, 2009. Establishment of a cross-European field site network in the ALARM project for assessing large-scale changes in biodiversity. *Environ Monit Assess* 164: 337-348.

Leistra M, 2005. *Estimating input data for computations on the volatilisation of pesticides from plant canopy and competing processes*. Alterra-rapport 1256, Alterra, Wageningen, The Netherlands, 80 pp.

Pistocchi A, Groenwold J, Lahr J, Loos M, Mujica M, Ragas A, Rallo R, Sala S, Schlink U, Strebel K, Vighi M, Vizcaino P, 2010. Mapping cumulative environmental risks: Examples from the EU NoMiracle Project. *Environ Model Assess* in press. DOI: 10.1007/s10666-010-9230-6.

Sala S and Vighi M, 2008. GIS-based procedure for site-specific risk assessment of pesticides for aquatic ecosystems. *Exotox Environ Saf* 69:1-12.

Sala S, Cavalli M, Vighi M, 2010. Spatially explicit method for ecotoxicological risk assessment of pesticides for birds. *Ecotox Environ Saf* 73: 213-221.

Sumpter JP, Johnson AC, Williams RJ, Kortenkamp A, Scholze M, 2006. Modelling effects of mixtures of endocrine disrupting chemicals at the river catchment scale. *Environ Sci Technol* 40: 5478-5489.

Vaj C and Vighi M, 2009. Assessing and mapping pesticide risk on ecosystems. III: soil community. Presented to: NO MIRACLE final conference "Multiple Stressors – Novel Methods for Integrated Risk Assessment", 28th – 30th September 2009, Århus, Denmark.

Verro R, Calliera M, Maffioli G, Auteri D, Sala S, Finizio A, Vighi M, 2002. GIS-based sysyem for surface water risk assessment of agricultural chemicals. 1. methodological approach. *Environ Sci Technol* 36: 1532-1538.

Vighi M and Calliera M, 1999. *Stima del rischio ambientale da prodotti fitosanitari: un caso di studio nella provincia di Forlì-Cesena*. Osservatorio Agroambientale – Centrale Ortofrutticola, Cesena (FC), Italy, 95 pp. In Italian.

Westphal C, Bommarco R, Carré G, Lamborn R, Morison N, Petanidou T, Potts SG, Roberts SPM, Szentgyörgyi H, Tscheulin T, Vaissière BE, Woyciechowski M, Biesmeijer JC, Kunin WE, Settele J, Steffan-Dewenter I, 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecol Monogr* 78: 653–671.

Williams IH, 1994. The dependences of crop production within the European Union on pollination by honey bees. *Agric Zool Rev* 6: 229–257.

CHAPTER V

Year-round monitoring of soil microarthropod community under pesticide application in a real case

Abstract

Plant Protection Products (PPP) applied in agricultural practices can have adverse effects on soil microarthropod communities. A year-round field survey was conducted in a vineyard in Northern Italy, for monitoring changes in the structure of this community under the application of pesticides, focusing on springtails and mites. A general decrease in abundances was recorded after the application of the insecticide chlorpyrifos. A recovery at the end of the productive season can be seen a few meters outside the field. Within the vineyard the recovery is reached at the beginning of the next spring. Using suitable multivariate statistical tools, the behaviour of each taxon in relation to the stressors was assessed and the organisms favoured or affected by them were identified. The role of physical and chemical stressors in defining the relative behaviour of the community is also discussed.

Keywords: microarthropods, pesticides, PRC, community structure.

5.1. Introduction

As shown in chapter III, plant protection products (PPP) applied on crops may have an adverse effect on non-target organisms, even in non-target compartments, that can be reached through transport mechanisms as drift. As highlighted, microartrhopods can be affected by the application of pesticides more than other non-target organisms, because of their sensitivity to this kind of chemicals. They are, with Anellida, the organisms most representative of a soil community. They play an important role in ecosystem services and are considered as pre-decomposers, because crushing organic debris, they makes easier the action of microorganisms. Moreover, through their movements into the compartment, they can contribute to the soil structuring at the micro-scale. Microarthropods contribute directly to the humus fraction and, even though their role in comminution and mixing may be small in comparison with that of larger invertebrates, they exercise important function in mineral turnover, vegetation succession, and as decomposers of organic matter (Buthcer *et al.*, 1971). The two major groups of soil microarthropods are springtails and mites: they are ubiquitous (they can be found from tropical forests to the Arctic and Antarctica) and can be representative of several trophic niches, from detritivores, to predators, to eating parts of plants and roots (Angelini *et al.*, 2002).
Microarthropods are widely applied as indicators, and are used into indexes of soil quality. The basis its that different groups of pedofauna respond in different ways to a perturbation or adverse conditions, taking into account that for some organisms mobility is strongly reduced. An index utilising the totality of the microarthropod community is the index of biological quality of soil (QBS-ar; Parisi, 2001), that is based on the adaptation of a taxon to the soil compartment. It is more related to the affinity of an organism to the compartment, than to taxonomy, but the major critic point is that it is not a specific indicator and doesn't give any information on the kind of stress in case of a negative result. An other index that uses soil organisms is the mite/springtail ratio (Bachelier, 1986) that is based on the assumption that if the biocenosis is at equilibrium the number of mites exceeds the number of springtails. As QBS-ar, also this index is not specific and doesn't give any information on the stressor.
In the environment, beside an anthropogenic contamination, also the physical parameters can act as stressors, with their normal fluctuations or extreme events, giving as result a combined stress. The response of populations to the extremely complex conditions that can be reached in field cannot be observed in laboratory studies that can mimic just a small part of the total complexity. Moreover, a community reacts to the stressors in function of the population responses and the interspecific relations. Also this point is difficult, if not impossible, to be reproduced in laboratory studies.

The need to move towards investigations in field is thus highlighted (see e.g. in the recent literature Vighi *et al*., 2006).

In this chapter a field investigation is reported, performed in the same field site used in the master case of NO MIRACLE (Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe) project of the European Commission. The chemical stress to which organisms are exposed is the presence of pesticide in the vineyard, described and assessed in chapter III.

5.2. Materials and methods

5.2.1. Sampling scheme

The activity was performed in the same site used for soil risk assessment, identified within the NO MIRACLE master case (see chapter III). It is a 5 ha vineyard located in the Meolo river basin (Veneto region, Northern Italy), partly cultivated with Pinot Grigio variety and partly with Prosecco plants. Three sampling stations were identified: one within the field (A), one 4 m away from the last row (B), in and close to Pinot Grigio cultivars, and one 10 m far from the last row of Prosecco cultivar (C) (figure 5.1).

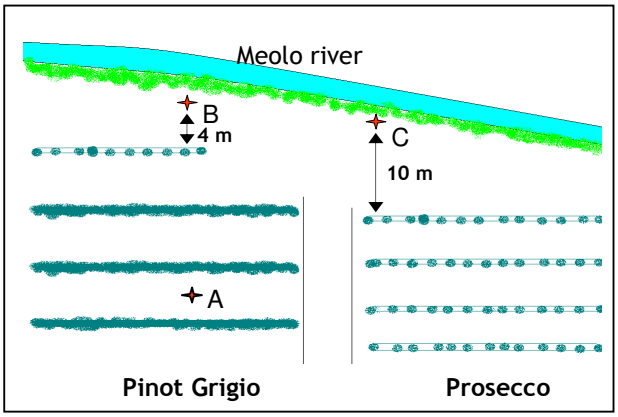

Figure 5.1. Field site scheme (modified after Vaj *et al.*, 2010).

The community was studied in a year-round sampling campaign from June 2008 to June 2009. The sampling dates were selected according to insecticide (thiamethoxam and chlorpyrifos) applications during the productive season and then at intervals in order to register the seasonal fluctuations of organisms (figure 5.2).

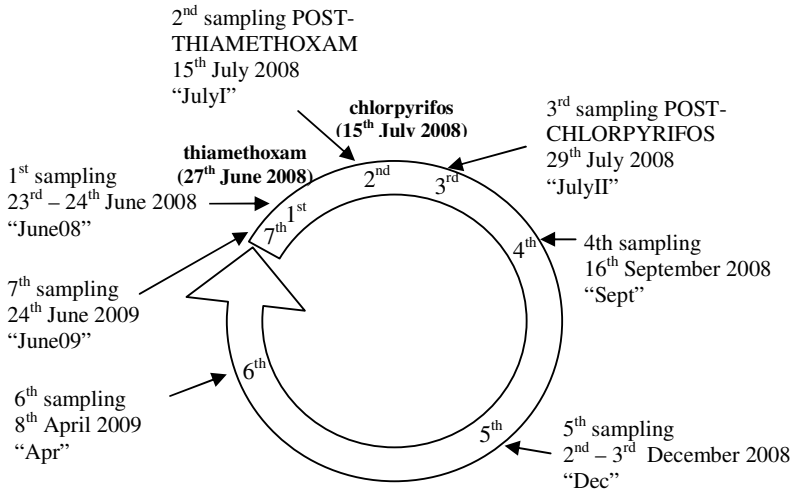

Figure 5.2. Sampling scheme used in the field study (modified after Vaj *et al.*, 2010), also indicating insecticide application dates and codes of the samplings between quotation marks.

The two insecticides applied are a neonicotinoid (thiamethoxam) and an organophospate (chlorpyrifos). Their physical-chemical, halflives and ecotoxicological properties were collected from official available information and are reported in table 5.1.

Table 5.1. Physical-chemical, halflives and ecotoxicological properties of the two insecticides (-: data not available).

	PHYSICAL-CHEMICAL PROPERTIES				
Active Ingredient	**MW**	**S (g/m3)**	**VP (Pa)**	**Log K_{OW}**	**DT_{50} soil (d)**
chlorpyrifos	350.6[(1)]	1.4[(1)]	2.70E-03[(1)]	5.261[(2)]	70[(2)]
thiamethoxam	291.7[(1)]	4100[(1)]	6.60E-09[(1)]	-0.13[(1)]	39[(3)]
	ECOTOXICOLOGICAL PROPERTIES				
	Acute LC_{50} (14 days *Eisenia foetida*) (mg/kg)		**LC_{50} *Typhlodromus pyri* (mg/kg)**		**LC_{50} (35 days) *Folsomia candida*(mg/kg)**
chlorpyrifos	210[(1)]		1.3[(3) *]		0.2[(3)]
thiamethoxam	1000[(1)]		-		-

(1) Tomlin, 2003; (2) Verro *et al.*, 2009; (3) FOOTPRINT, 2006; * LC_{50} (mg/kg) value derived from LR_{50} (g/ha) value.

Some soil physical parameters were measured. Temperature can have an influence on belowground community distribution and behaviour (Hutha and Hänninen, 2001; Choi *et al.*, 2002, Malmström, 2008), thus during each

sampling event 2 cm depth temperature was measured in field using a mercury thermometer. Also soil moisture is considered to have an influence (Hutha and Hänninen, 2001; Choi *et al.*, 2002). For sampling dates from June 2008 and September 2008 soil moisture was estimated starting from the empirical equation derived from USDA texture triangle (Soil Survey Division Staff, 1993). The equation (eq. 5.1) calculates moisture equivalent, assumed nearly equal to field capacity, from sand, silt and clay percentages.

$$ME = 0.023 \cdot Sa + 0.25 \cdot Si + 0.61 \cdot Cl \qquad \text{(Eq. 5.1)}$$

where:
ME (%) is moisture equivalent (the value will be converted in g/kg for further analysis);
Sa (%) is sand (2-0.05 mm) percentage;
Si (%) is silt (0.05-0.0002 mm) percentage;
Cl (%) is clay (<0.0002 mm) percentage.

ME values were calculated for the three sampling stations (344 g/kg for A point, 268 g/kg for B point and 232 for C point). Through direct observations in field and the aim of experts judgment, for each sampling date the ratio with the field capacity was assessed (%) and the moisture was calculated (g/kg). Table 5.2 explains the procedure.
The same calculation was made for a reference field situation (December 2008 sampling, under rain, wet soil) and error never exceeded 20%.

Table 5.2. Calculation scheme for assessing moisture from June 2008 to September 2008 in the three sampling stations.

QUALITATIVE FIELD OBSERVATIONS			**CALCULATED HUMIDITY**		
Date code	**Observation**	**% with ME**	**A (g/kg)**	**B (g/kg)**	**C (g/kg)**
June08	Fairly dry	27	93	72	63
JulyI	Dry	25	86	67	58
JulyII	Very dry	23	79	62	53
Sept	Moist	60	207	161	139

This is a very rough *a posteriori* estimation. Suitable models for assessing soil moisture for these 4 dates could be used as refinement of this step.
Soil samples from the three points were analysed for soil density (from 5 replicates), USDA classification texture (sieving and sedimentation method), organic carbon content (Walkley & Black potassium dichromate method) and pH in H_2O. The measurements in lab were performed for one sampling date and those parameters are considered not to vary noticeably throughout the year. Organic carbon and pH variations among the three points can be considered as negligible. All soil parameters are reported in table 5.3.

In B and C points a possible physical stress could be given by the passage of agricultural vehicles, that is not quantifiable. In contrast, in A point all the samples were taken in the middle of surface between two rows, while the wheels press down a portion of soil closer to plant rows.

Table 5.3. Soil parameters measured in field or in lab for the three sampling stations.

Site	Date code	T (°C) at 2 cm depth	Soil moisture (g/kg)	Soil density (g/cm^3)	Texture	OC content (g/kg)	pH
A	June08	24	93.0	0.760	Silty loam	28	7.4
	JulyI	29	86.1				
	JulyII	29	79.2				
	Sept	23	206.6				
	Dec	8	256.0				
	Apr	19	250.3				
	June09	23	209.5				
B	June08	30	72.4	0.772	Loam	27	7.4
	JulyI	29	67.1				
	JulyII	29	61.7				
	Sept	23	160.9				
	Dec	8	283.0				
	Apr	21	258.5				
	June09	24	209.4				
C	June08	29	62.7	0.834	Loam	27	7.3
	JulyI	29	58.0				
	JulyII	30	53.4				
	Sept	22	139.3				
	Dec	8	248.0				
	Apr	19	282.9				
	June09	22	216.3				

5.2.2. Microarthropods sampling and identification

Microarthropods were sampled according to ISO/FDIS 23611-2 (ISO, 2006) guidelines using a split corer of 10 cm diameter up to 10 cm depth. Samples were collected in replicates and the animals were extracted with Berlese method at University of Milano-Bicocca, Milano (Italy) or modified Tullgren-Berlese method at VU University, Amsterdam (The Netherlands) and kept in a preservative solution (alcohol, acetic acid and formaldehyde 1000:30:3).

Sampled organisms were identified to the order level (Chinery, 1998; Angelini *et al*., 2002; Codurri *et al.*, 2005), except the most abundant taxa. Some were identified to the family level, ants and springtails (Hopkin, 2007; Bellinger *et al.* 1996-2010), while mites were divided into the four major groups (Astigmata, Cryptostigmata, Mesostigmata e Prostigmata) according to the literature (e.g. Koolhaas *et al.*, 2004).
At least four replicates per sample underwent identification.

5.2.3. Statistical analyses

For the analysis of the results statistical tools were used.
Analysis of variance (One-way ANOVA with a code taking into account both point and date as factor) was performed using Minitab 15.1.30.0.

Biodiversity of samples was assessed using two different indexes, proposed by Simpson (1949) and Shannon and Weaver (1963).
Simpson's index (*C*) is commonly used for biodiversity measuring and is expressed by equation 5.2:

$$C = \sum \left(\frac{n_i}{N} \right)^2 \qquad \text{(Eq. 5.2)}$$

where:
n_i is the abundance of individuals of the i-th taxon;
N is the total number of individuals in the sample.

C ranges from 0 to 1 and assumes higher values when just a few species or taxa dominate the community, thus lower values indicate higher biodiversity.

Shannon-Wiener's index (*H'*) is expressed by equation 5.3:

$$H' = -\sum_{i=1}^{S} p_i \cdot \ln p_i \qquad \text{(Eq. 5.3)}$$

where:
S is the total number of species or taxa;
p is the proportion of individuals of the i-th taxon in the community, $p_i = n_i/N$.

H' ranges theoretically from 0 to ∞, and the highest is the value, the highest is the biodiversity.

Multivariate statistical analysis was also performed: Principal Component Analysis (PCA; Jolliffe, 1986; Jackson, 1991; Todeschini, 1998) using XLSTAT 4.0 and Principal Response Curves (PRC; Van den Brink e Ter Braak, 1998; 1999; Van den Brink *et al*, 2009) using Canoco for Windows 4.5. This last method is very interesting in investigations on biological communities, because it takes into account weights given by different taxa in the time trend of the community, referred to a control. Briefly, it is based on a redundancy analysis and uses time as x-axis, forcing the control point to 0 for each date. The values given to the other samples will be based on the distance to the control, weighted for each taxon present inside the sample and its variation, positive or negative, compared to the control. Taxa with a high weight (B_k) value are those affected by the treatment or the stressor, those with low B_k value are favoured by treatment or stressor (because of indirect effects or less vulnerability to the stressor). B_k is given to each taxon by the statistical tool starting from the dataset and it is not related to a real general situation. This could be a bias in the analysis that will not take into account the variation of rare species, unlike other kind of analysis as e.g. SPEAR method developed for the freshwater compartment (Liess and von der Ohe, 2005)

Prior the multivariate analysis data were transformed using a range scaling (eq.5.4):

$$x'_{ij} = \frac{x_{ij} - L_j}{U_j - L_j} \qquad \text{(Eq. 5.4)}$$

where:
x'_{ij} is the new variable;
x_{ij} is the original variable;
L_j is the minimum value among the variables of the j-th row;
U_j is the maximum value among the variables of the j-th row.

The new values are thus ranging between 0 and 1.

5.2.4. Calculation of exposure

Pesticide exposure was calculated starting from the detailed information on active ingredients applied in the vineyard. In table 5.4 all the active ingredients used in the vineyard and the total amount applied are reported.

Table 5.4. Active ingredients and total amounts applied in the vineyard. For repeated applications the first and the last dates in the productive season are given. Italics: a.i. applied only on Pinot Grigio variety.

a.i.	Application dates		Total amount (kg)
	start	end	
sulfur	28-Apr-08	11-Aug-08	364.00
	15-Apr-09	22-Jun-09	172.00
mancozeb	28-Apr-08	20-Jun-08	82.50
dimethomorph	19-May-08	27-Jun-08	5.00
glyphosate	20-May-08	5-Aug-08	11.65
	20-Apr-09	14-Jun-09	7.20
oxadiazon	20-May-08		3.70
folpet	24-May-08	14-Jun-08	54.00
	15-Apr-09	12-Jun-09	44.95
iprovalicarb	14-May-08	14-Jun-08	0.75
cyprodinil	17-Jun-08		1.05
	12-Jun-09		1.05
fludioxonil	17-Jun-08		0.70
	12-Jun-09		0.70
thiamethoxam	27-Jun-08		0.25
	22-Jun-09		0.25
copper oxychloride	27-Jun-08	5-Aug-08	24.00
	12-Jun-09	22-Jun-09	8.69
chlorpyrifos	15-Jul-08		2.25
copper sulphate	22-Jul-08	11-Aug-08	8.94
mepanipyrim	5-Aug-08		1.00
*thiophanate-metyl**	7-Jun-09		1.26

* Partly degrades in soil into carbendazim.

The entire period was divided into three sub-periods: productive season 2008, October 2008-April 2009 and the beginning of the productive season 2009.

For productive seasons 2008 and 2009 the amount of insecticides and fungicides that reaches soil within the field was calculated subtracting crop interception percentages from the application rates. Additional inputs to soil from foliage wash-off was taken into account according to Leistra (2005). For sampling points outside the field drift models (Ganzelmeier *et al.*, 1995 modified by Barmaz, 2009) were applied. Herbicides are applied directly at the ground level, thus crop interception and drift transport are considered as negligible.

Pesticides concentrations were diluted in 10 cm deep layer and converted in mass/soil mass unit using soil density values measured in lab. Kinetic degradation curves were constructed for all the active ingredients. The depth

of 10 cm was selected because it is the layer of major density of the microarthropod community (Van Gestel, personal communication). Therefore it was also the depth of soil samples.
From October 2008 and the middle of April 2009 only leaching was taken into account and calculated according to Bolt and Bruggenwert (1976), assuming in this case a trigger depth (*Xp*) of 10 cm for chemical transport.
Detailed information on this procedure are reported in chapter III, section 3.2.3.

For a better assessment of the exposure to which organisms are subjected before a sampling event, Time Weighted Average (TWA) was calculated for a time interval of 14 days between insecticide applications and post-application samplings (eq. 5.5).

$$TWA = \int_{t_0}^{t} C_0 e^{-kt} dt = \frac{\left(C_0 - C_0 e^{-kt}\right)}{kt} = \frac{\left(C_0 - C_t\right)}{kt} \qquad \text{(Eq. 5.5)}$$

where:
C_0 is the initial concentration of the chemical, at t_0;
C_t is the concentration of the chemical after a given time t;
t_0 is the initial time;
t is the time when the calculation is stopped;
k is the constant of the first order kinetic degradation curve and is calculated as $ln2/DT_{50}$.

The results of the calculation are reported in table 5.5.

Table 5.5. TWAs calculated for each point in each sampling date (na: not applied; nd: not drifted; neg: negligible value, <0.0001 mg/kg soil).

TWA 14 d 10 cm (mg/kg soil)	JUNE08			JULYI		
	A	B	C	A	B	C
mancozeb	0.224	0.009	0.004	0.170	neg	neg
sulfur	16.870	0.963	0.428	18.147	1.076	0.478
dimethomorph	0.749	0.025	0.011	0.690	0.028	0.012
glyphosate	1.139	nd	nd	1.328	nd	nd
oxadiazon	0.858	nd	nd	0.776	nd	nd
folpet	2.116	0.076	0.034	0.225	0.003	0.001
iprovalicarb	0.080	0.003	0.001	0.057	0.002	0.001
cyprodinil	0.162	0.008	na	0.176	0.012	na
fludioxonil	0.102	0.005	na	0.073	0.005	na
thiamethoxam	na	na	na	0.046	0.002	0.001
copper oxychloride	na	na	na	1.674	0.110	0.049
chlorpyrifos	na	na	na	0.148	0.011	0.005
copper sulphate	na	na	na	na	na	na
mepanipyrim	na	na	na	na	na	na
thiophanate-methyl	na	na	na	na	na	na
carbendazim	na	na	na	na	na	na
TWA 14 d 10 cm (mg/kg soil)	**JULYII**			**SEPT**		
	A	**B**	**C**	**A**	**B**	**C**
mancozeb	0.003	neg	neg	neg	neg	neg
sulfur	19.034	1.125	0.500	14.468	0.715	0.318
dimethomorph	0.651	0.022	0.010	0.311	0.010	0.005
glyphosate	1.130	nd	nd	1.419	nd	nd
oxadiazon	0.728	nd	nd	0.581	nd	nd
folpet	0.238	neg	neg	0.094	neg	neg
iprovalicarb	0.043	0.001	0.001	0.007	neg	neg
cyprodinil	0.138	0.010	na	0.059	0.004	na
fludioxonil	0.042	0.003	na	0.006	neg	na
thiamethoxam	0.046	0.002	0.001	0.019	0.001	neg
copper oxychloride	2.959	0.174	0.077	5.441	0.226	0.101
chlorpyrifos	0.283	0.020	0.009	0.174	0.012	0.005
copper sulphate	0.508	0.015	0.007	2.313	0.083	0.037
mepanipyrim	na	na	na	0.389	0.015	na
thiophanate-methyl	na	na	na	na	na	na
carbendazim	na	na	na	na	na	na

Table 5.5 – continued.

TWA 14 d 10 cm (mg/kg soil)	DEC			APR		
	A	B	C	A	B	C
mancozeb	neg	neg	neg	neg	neg	neg
sulfur	2.628	0.121	0.054	0.143	0.006	0.003
dimethomorph	neg	neg	neg	neg	neg	neg
glyphosate	0.576	nd	nd	n	nd	nd
oxadiazon	neg	nd	nd	neg	nd	nd
folpet	neg	neg	neg	neg	neg	neg
iprovalicarb	neg	neg	neg	neg	neg	neg
cyprodinil	neg	neg	na	neg	neg	na
fludioxonil	neg	neg	na	neg	neg	na
thiamethoxam	neg	neg	neg	neg	neg	neg
copper oxychloride	5.802	0.225	0.100	5.751	0.223	0.099
chlorpyrifos	0.021	0.001	0.001	neg	neg	neg
copper sulphate	2.236	0.080	0.036	2.117	0.076	0.034
mepanipyrim	neg	neg	na	neg	neg	na
thiophanate-methyl	na	na	na	na	na	na
carbendazim	na	na	na	na	na	na

TWA 14 d 10 cm (mg/kg soil)	JUNE09		
	A	B	C
mancozeb	na	na	na
sulfur	13.114	0.798	0.355
dimethomorph	na	na	na
glyphosate	0.996	nd	nd
oxadiazon	na	na	na
folpet	0.830	0.035	0.016
iprovalicarb	na	na	na
cyprodinil	0.111	0.008	na
fludioxonil	0.066	0.005	na
thiamethoxam	0.030	0.001	0.001
copper oxychloride	6.386	0.259	0.115
chlorpyrifos	neg	neg	neg
copper sulphate	2.048	0.074	0.033
mepanipyrim	na	na	na
thiophanate-methyl	0.135	0.004	na
carbendazim	0.144	0.009	na

5.3. Results and discussion

5.3.1. Main trends in soil community

The results presented in this section have been converted in individuals/m^2. Standard deviations were calculated, giving very high results. This is not surprising: soil compartment usually shows high variations of properties, concentrations of substances, etc. because the movements in the matrix are not easy as in an homogenous compartment (e.g. water). Moreover soil organisms tend to cluster and live aggregated, as can be found in other works available in the literature (e.g. Koolhaas *et al.*, 2004).

Figure 5.3 shows the time trend of the mean counts of the sampled organisms of the whole community. Statistically significant differences in soil community counts were found, using ANOVA and are listed below.
For each point, only the couples of dates with statistically significant differences are reported:

- A point: Sept-Dec ($P<0.05$);
- B point: JulyII-Sept ($P<0.05$), Sept-Dec ($P<0.01$), June08-Sept ($P<0.05$);
- C point: JulyII-Sept ($P<0.05$), July08-Sept ($P<0.05$).

For each date, only the couples of points with statistically significant differences are reported:

- June08: no statistically significant differences;
- JulyI: no statistically significant differences;
- JulyII: no statistically significant differences;
- Sept: A-B ($P<0.01$), A-C ($P<0.05$);
- Dec: A-B ($P<0.05$), A-C ($P<0.05$);
- Apr: no statistically significant differences;
- June09: A-C ($P<0.05$).

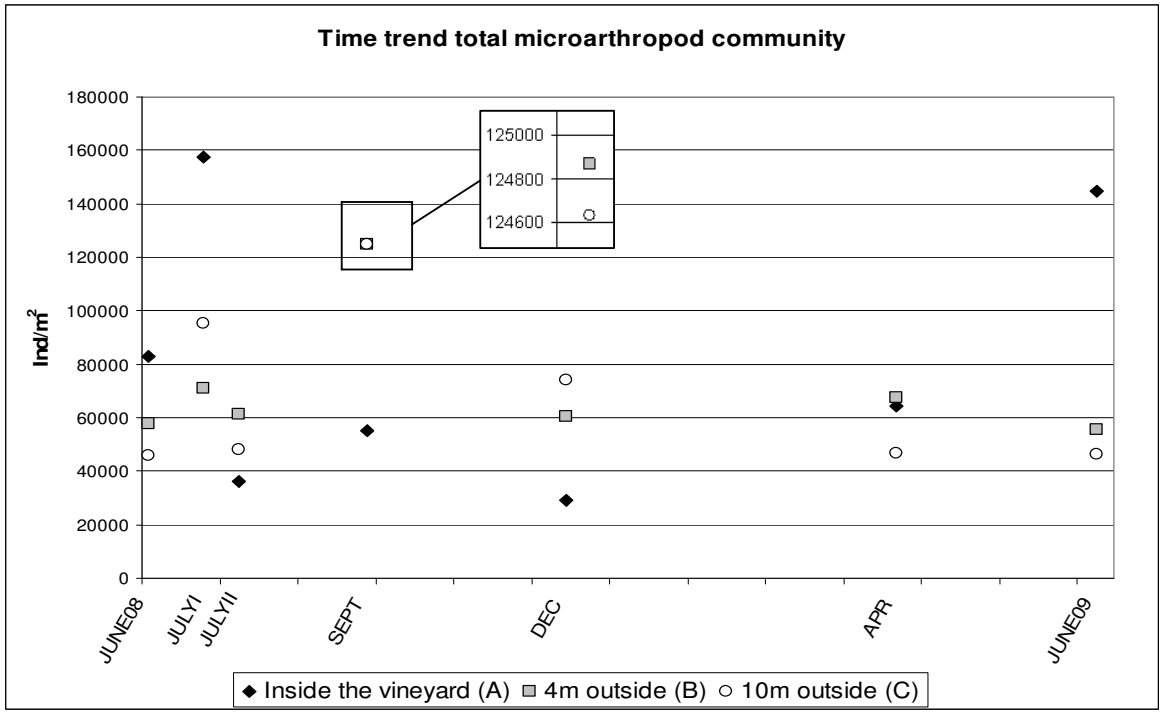

Figure 5.3. Time trend of the total microarthropod community sampled in the three stations, A, B and C, as mean values of the replicates. In the small square the detail of the abundance of the community in B and C points is shown.

Although the most of the differences were not statistically significant, some considerations can be done. An indication of decrease in total abundance seems to be recorded between JulyI and JulyII, when the insecticide chlorpyrifos is applied. Although the differences are not significant, the decrease seems to be higher inside the vineyard, where the a.i. undergoes direct fallout, than outside. Afterwards community recovers in abundance in September outside the vineyard, but not inside (statistically significant difference). In December abundances are fairly low in all the three points, due to the adverse conditions of the season, even the soil is not yet frozen. In A point the organisms are less than in the other two stations. Thus before winter within the field the community did not show any recovery. In April, at the beginning of the spring, numbers are comparable among the three stations, but not significantly different from December. At the end of the investigations the situation is comparable to the beginning (June 2008).
Since most of the differences among samples were not statistically significant, it can be concluded that maybe the sample size was inadequate. Anyway, it should be noted that in defining the sample size also time of analysis and costs have to be taken into account.

In figure 5.4 main trends of the mean numbers of the springtails community are shown. Springtail families were bunched into major groups: Poduromorpha (Hypogastruridae, Neanuridae, Onychiuridae and Tullbergiidae), Isotomidae, Entomobryidae, Symphypleona (Bourletiellidae, Katiannidae, Sminthuridae and Sminthurididae) and Neelipleona (Neelidae).

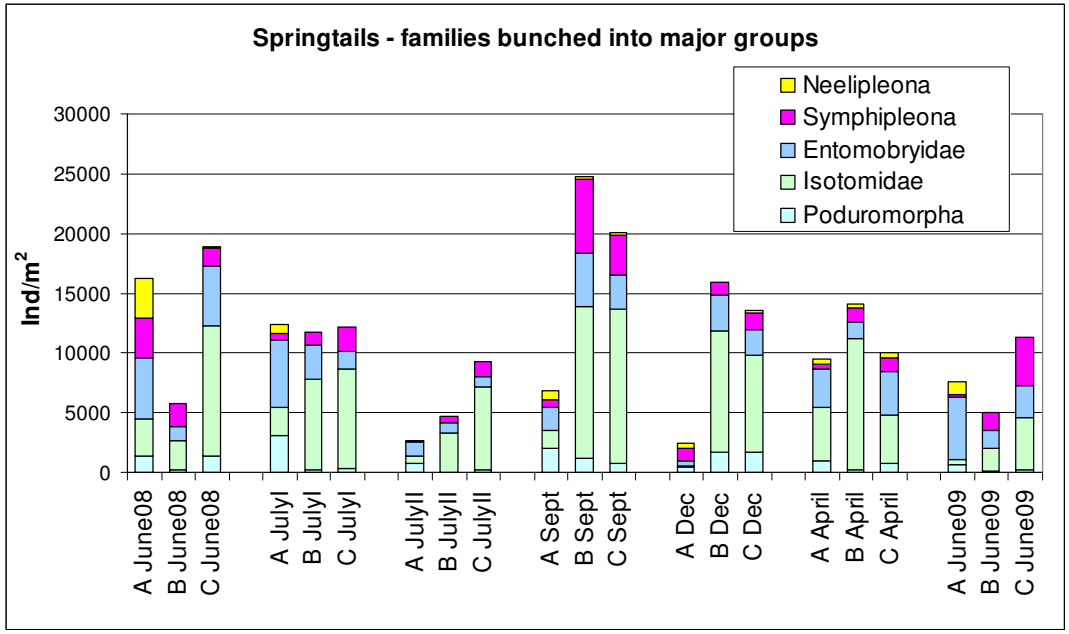

Figure 5.4. Time trend of total mean number of springtails sampled in the three stations, A, B and C, bunched into major groups (modified after Vaj *et al.*, 2010)

Statistically significant differences in counts were found, using ANOVA and are listed below.
For each point, the couples of dates with statistically significant differences in total numbers of Collembola are reported.

- A point: JulyI-JulyII (P<0.01), Dec-Apr (P<0.01);
- B point: JulyII-Sept (P<0.01), June08-Sept (P<0.01);
- C point: no statistically significant differences.

For each date, couples of points with statistically significant differences in total numbers of springtails are listed:

- June08: no statistically significant differences;
- JulyI: no statistically significant differences;
- JulyII: no statistically significant differences;
- Sept: A-B (P<0.01);
- Dec: A-B (P<0.01);
- Apr: no statistically significant differences;
- June09: no statistically significant differences.

Since sometimes statistically significant differences were due to variations of particular groups, differences in counts are reported also for individual groups. Only couples of dates or points with P<0.05 or P<0.01 are listed for each group.

- Poduromorpha:
  - A point: JulyI-JulyII (P<0.05);
  - JulyI: A-B (P<0.01), A-C (P<0.01);
  - Apr: A-B (P<0.05).
- Isotomidae:
  - A point: Dec-Apr (P<0.01), Apr-June09 (P<0.01);
  - JulyI: A-C (P<0.01);
  - Dec: A-B (P<0.01).
- Entomobryidae:
  - A point: JulyI-JulyII (P<0.05);
  - JulyI: A-C (P<0.05);
- Symphypleona:
  - B point: JulyII-Sept (P<0.05);
  - C point: Apr-June09 (P<0.05);
  - JulyII: A-C (P<0.05);
  - Sept: A-B (P<0.05);
  - June09: A-C (P<0.01).
- Neelipleona:
  - B point: JulyII-Sept (P<0.05), June08-Sept(P<0.05);
  - C point: Apr-June09 (P<0.05);
  - Dec: A-B (P<0.05).

Total number of springtails decreases between the two samplings in July, when chlorpyrifos is applied, inside the field (A: $P<0.01$). The result is in accordance with Frampton (2000) that found large differences in counts of several species of Collembola after the use of chlorpyrifos. There is an increase in the total numbers in September outside the field (B: $P<0.01$; C: only indications, no statistically significant), but a recovery inside the vineyard does not happen.
Focusing on groups, Poduromorpha and Entomobryidae seem to be affected by chlorpyrifos application ($P<0.05$ in a point for the couple JulyI-JulyII).
Isotomidae have low numbers inside the vineyard, compared to the other two points (in JulyI and Dec $P<0.01$, for other dates only indications without significant differences), except in April, when their number is comparable to those found 10 m away from the field. This trend could be explained by pesticide presence inside the vineyard to which this family could be more vulnerable. Indeed in April PPPs are not yet applied on vineyard and the community is starting a new annual cycle.
Symphypleona seem to be affected by pesticide stress acting inside the vineyard, because they are found in lower numbers in A point compared to the other two in JulyII, Sept and June09 ($P<0.05$, $P<0.05$ and $P<0.01$ respectively). Indeed, in these dates a mixture of PPP is present in the soil in A point.
On the other hand, Neelipleona seem to be present in A point, except immediately after chlorpyrifos application, but not in B and C (only in December and April, but in low numbers). This could be an indirect effect of the absence of other taxa affected by pesticide presence inside the vineyard and a consequence of some traits that make Neelipleona less vulnerable to PPP presence. Due to the low numbers this trend cannot be supported by statistically significant differences, hence it should be seen as an indication. Similar observations were possible also for two Poduromorpha families, Hypogastruridae and Tullbergiidae (for further discussion about these families see figure 5.11).

Time trend of mites divided into the four major groups for the whole year is shown in figure 5.5.

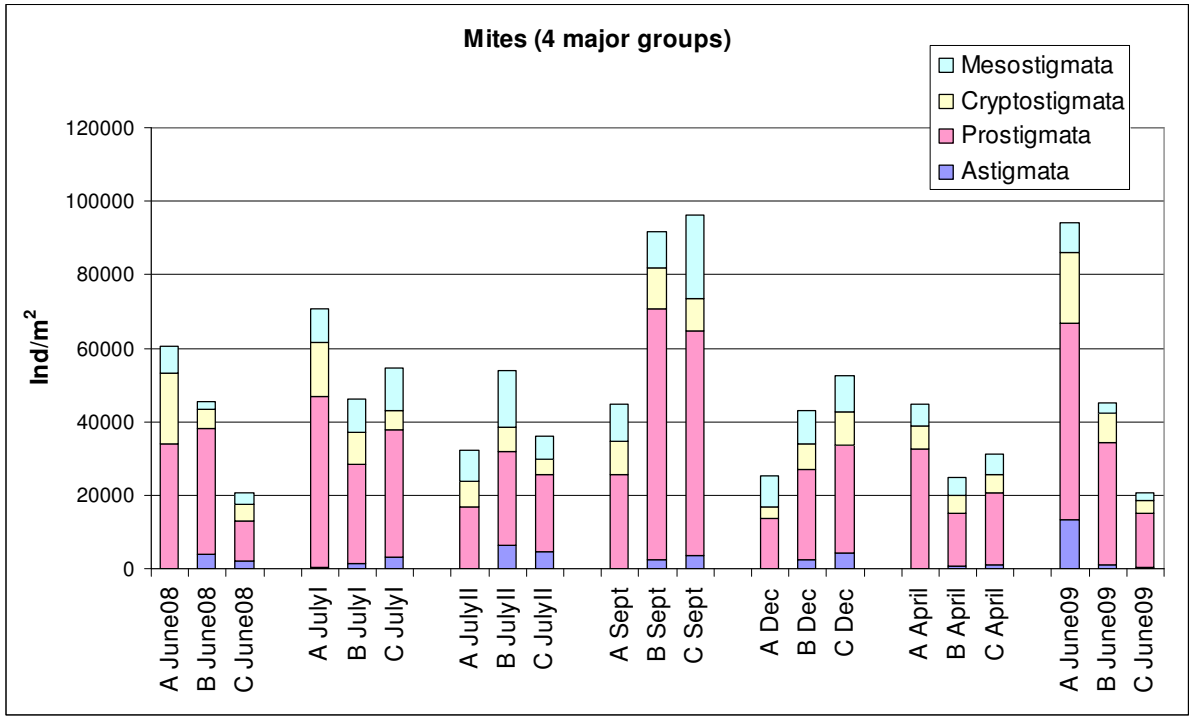

Figure 5.5. Time trend of total mean number of mites sampled in the three stations, A, B and C, divided into the four major groups (modified after Vaj *et al.*, 2010)

Statistically significant differences in counts were found, using ANOVA and are listed below.
For each point, the couples of dates with statistically significant differences in total numbers of mites are reported.

- A point: Sept-Dec ($P<0.05$);
- B point: Sept-Dec ($P<0.05$);
- C point: June08-JulyI ($P<0.01$) and June08-Sept ($P<0.05$).

For each date, couples of points with statistically significant differences in total numbers of mites are listed:

- June08: no statistically significant differences;
- JulyI: no statistically significant differences;
- JulyII: no statistically significant differences;
- Sept: A-B ($P<0.05$);
- Dec: no statistically significant differences;
- Apr: no statistically significant differences;
- June09: no statistically significant differences.

As for Collembola, differences in counts are reported also for individual groups. Only couples of dates or points with $P<0.05$ or $P<0.01$ are listed for each group.
- Astigmata:
  - JulyI: A-B ($P<0.05$);
  - Dec: A-B ($P<0.05$);
  - Apr: A-C ($P<0.05$).
- Prostigmata:
  - A point: JulyI-JulyII ($P<0.05$), Sept-Dec ($P<0.05$);
  - B point: JulyII-Sept ($P<0.05$);
  - C point: June08-JulyI ($P<0.01$), JulyII-Sept ($P<0.05$), June08-Sept ($P<0.05$);
  - Sept: A-B ($P<0.05$), A-C ($P<0.05$).
- Cryptostigmata:
  - A point: Sept-Dec ($P<0.01$);
  - B point: June08-Sept ($P<0.05$);
  - JulyI: A-B ($P<0.05$)
- Mesostigmata:
  - C point: Apr-June08 ($P<0.05$).

From figure 5.5 a decrease in mite numbers between the two sampling dates in July (after chlorpyrifos application) inside the vineyard cannot be clearly seen because differences are not significant. Anyway, some indications of a decreasing in A point and a subsequent recovery of the community in September, as happens for springtails, seem to be present, but confirmations are needed.

Even if without a statistic significance, at the beginning of the investigation the community seems to have higher numbers in A point compared to B and C, in a decreasing gradient from inside to outside the field. The same situation seems to be found one year later, in June 2009. Since inside the vineyard a chemical stressor is present (fungicides and herbicides applied at the beginning of the productive season), it can be hypothesized that, if the trend would be confirmed, the distribution can be driven by physical stressors, more than chemical, that can act outside the field. The stress could be addressed to the shade given by plants in A point or the passage of agricultural vehicles in B and C points. Soil density increases from A to B to C, as shown in table 5.3. These three differences could act as a driving forces, but only when insecticides are not present, because in other months mites trend seems to be different or even opposite as in June. Maybe the presence of insecticides, especially chlorpyrifos shadows the effect of the differences in the soil parameters.
Astigmata in some months are absent or sporadically present (one or two individuals per sample) inside the vineyard, but present outside (in some months the differences are significant): they could be more vulnerable to the PPPs applied on the crop that can persist throughout the year, at least until winter, or physical stressors present only inside the field.

Biodiversity was assessed using two indexes, Simpson (figure 5.6) and Shannon-Wiener (figure 5.7).

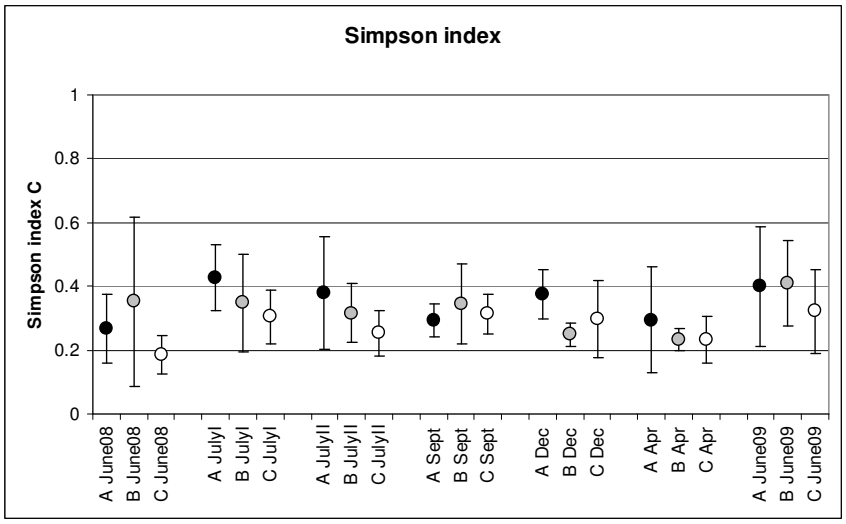

Figure 5.6. Mean Simpson index values measured for each point each sampling date. Biodiversity is higher for lower values. Intervals represents standard deviations.

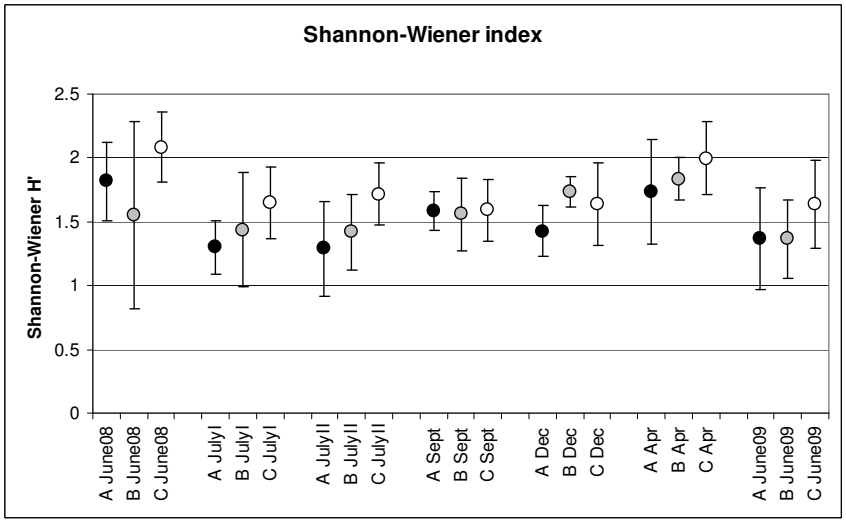

Figure 5.7. Mean Shannon-Wiener index values measured for each point each sampling date. Higher values mean higher biodiversity. Intervals represents standard deviations.

Results didn't show great differences in space or time. Anyway, these are two simple indexes than cannot report the changes in the structure of this kind of community. Further analysis have thus been done and are reported in the following sections.

5.3.2. Principal Response Curves

For investigating the effects on the structure of the community, Principal Response Curves (PRC) were constructed, using C point, the farthest from the vineyard, as control. The significance of the PRCs was tested by Monte Carlo permutation tests, by permuting whole time series in the partial RDA from which the PRC was obtained, using an F-type test statistic based on the eigenvalue of the component. All the PRCs performed showed $P<0.05$.
Figure 5.8 shows PRC constructed for the whole community.

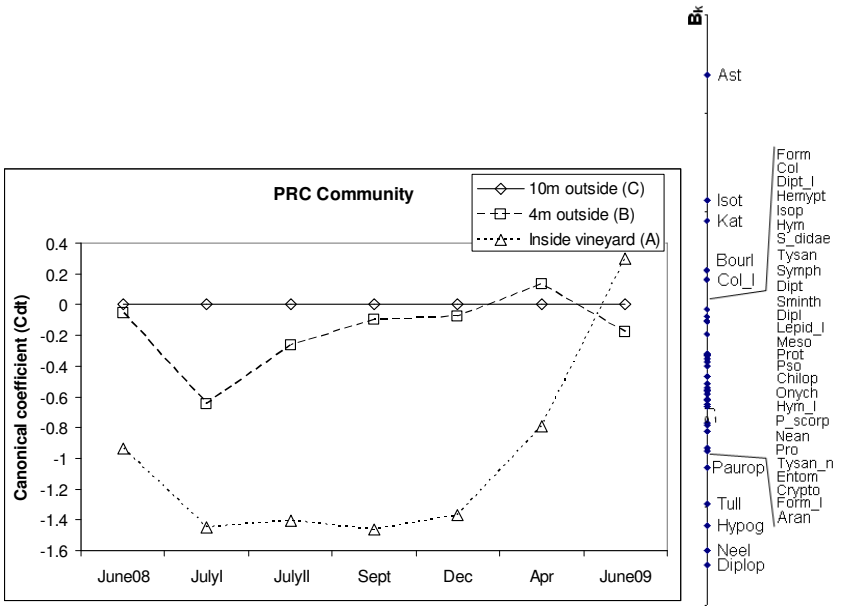

Figure 5.8. Principal Response Curves (PRC) of microarthropod community and weight axis (B_k) given to each taxon (for abbreviations see Appendix A).

The general trend shown in figure 5.8 is of course different from figure 5.3, because PRCs are not constructed using a normal mean, but a weighted mean. A point is far from the control even at the beginning of the investigation, while B point is closer to the control, except in one date, JulyI. Distance between A and control point after chlorpyrifos application does not increase, because a general decrease is recorded for all the three points for JulyII date (see figure 5.3). However, it should be noted that distance A-C does not decrease as well, because of the differences in the structure of the communities in the two different points. The community inside the vineyard is comparable to the others two points only at the end of the investigation, showing a sort of ideal recovery of the structure.

In B_k axis the taxa affected or favoured by stressors are shown. The methods considers astigmatid mites, three families of Collembola (Isotomidae, Katiannidae and Bourletiellidae) and Coleoptera larvae as affected, while pauropods, diplopods and other three springtail families (Tullbergiidae, Hypogastruridae and Neelidae) as favoured by stressors. For Isotomidae, Neelidae (the only family in the Neelipleona group) and Astigmata PRC trends confirm what observed in the time trend analysis of springtails and mites (section 5.3.1, figures 5.4 and 5.5 respectively).

PRCs were constructed for springtail community only (figure 5.9).

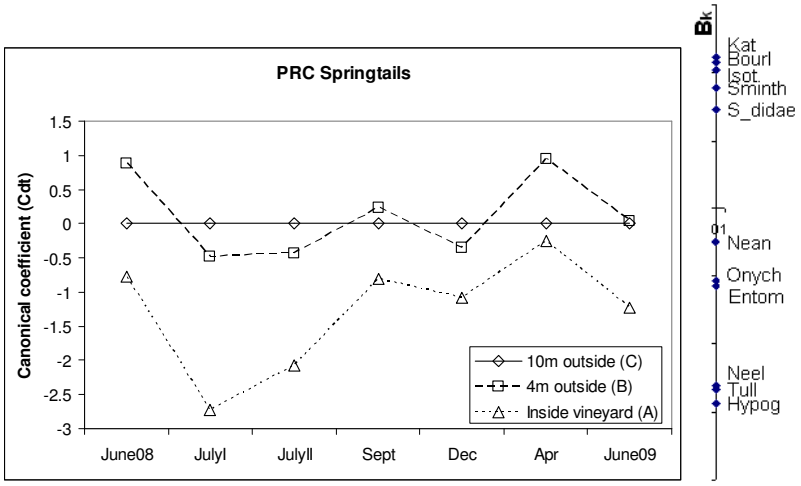

Figure 5.9. Principal Response Curves (PRC) for springtail community and weight (B_k) values given to each taxon. For abbreviations see Appendix A.

Community trend in A point is always the farthest from the control, while B point shows some differences, but is substantially similar to control. Only in Jun08 and April the structure of the community in B point is different compared to control, for the presence of taxa favoured by stressors. A point is far from the control in JulyI, while distance decrease after chlorpyrifos application (JulyII). This is due to the general decrease of the springtails community after chlorpyrifos application, recorded for all the three points. A final recovery of the structure inside the vineyard is not achieved, comparing to the control, but comparing to the situation one year before (June08).
Families affected or favoured by stressors are the same than in PRCs of the whole community (figure 5.8), with some small ordinal changes due to the absence of other taxa: Katiannidae, Bourletiellidae, Isotomidae, Sminthuridae and Sminthurididae are affected, while Neelidae, Tullbergiidae and Hypogastruridae are favoured. For Isotomidae, Neelidae, Tullbergiidae and Hypogastruridae previous considerations are confirmed. In a study by

Wiles and Frampton (1996) a Sminthuridae species (*Sminthurus viridis*) has been shown to be more susceptible to chlorpyrifos than three Isotomidae (*Folsomia candida*, *Isotomurus palistris* and *Isotoma viridis*). In our site specific analysis the relation is reversed, but it should be noted that the community is affected by a mixture of active ingredients, not only chlorpyrifos.

Figure 5.10 shows PRCs for mite community.

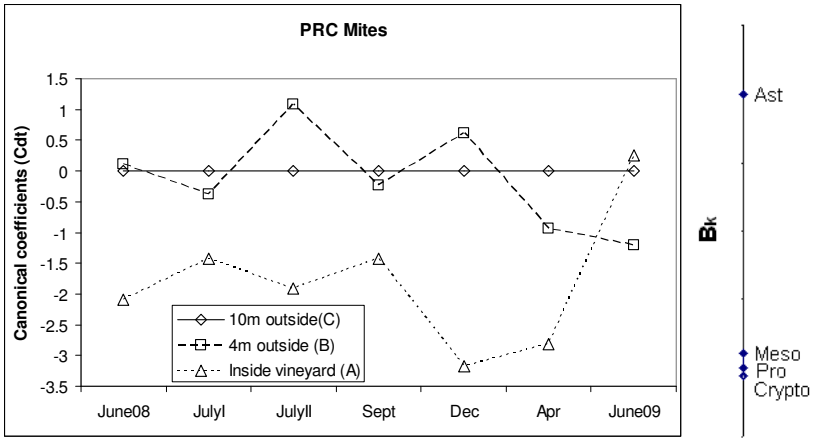

Figure 5.10. Principal Response Curves (PRC) for mite community and weight (B_k) values given to each taxon. For abbreviations see Appendix A.

The community in A point is different from the control since the beginning of the analysis, and this is due to the absence of Astigmata in this point (see also section 5.3.1, figure 5.5). The major decrease is in December and April, while in June09 the difference with the control is negligible. It should be noted that astigmatid mites are not present in C point in that date, thus it is more the control being similar to the vineyard site than the ideal recovery reached. The community structure seems not to be affected by insecticide applications, since in A point differences among the dates from June08 and September are not recorded.
Only the Astigmata group is affected by the stressors (not only insecticides), as shown in previous considerations (figure 5.8 and section 5.3.1 figure 5.5). The others three groups show an opposite relation with stressors.

Weight values (B_k) are dependent to the taxa pool used in the analysis, thus a quantitative comparison of B_ks given in the three different figures (from 5.8 to 5.10) is not possible.

5.3.3. Analysis of the community in relation to stressors

Principal Component Analysis (PCA) was used to analyse together the behaviour of the community with the possible stressors. PPPs values were expressed as TWAs calculated in the 14 days period before sampling, as explained in section 5.2.4, values reported in table 5.5). Soil properties used in the analysis were only those changing in space or time: soil moisture, density and temperature measured at 2 cm depth (values shown in table 5.3). Organic carbon and pH show negligible differences between inside and outside the field and are not considered to vary throughout the year. As input also the dataset of all the counts of samples for each date were used, not mean values. All the input value were range scaled using equation 5.4.
Two different PCAs were performed using chemical TWAs, community abundances, including and excluding physical parameters, for identifying the main stressors in this field work. Variance explained is higher using also physical parameters. Anyway, physical parameters are in the opposite part of the axis 1 (the one explaining the highest percentage of variance) respect of chemical contamination, but the distribution of the community on axis 1 does not change substantially in the two PCAs. Table 5.6 reports the ordination of variables according to axis 1 for both the PCAs. The only data coordinates that show some changes between the two PCAs are those of Astigmata and inorganic chemicals. Using also the physical parameters, the coordinates with negative values are very close each others, thus a real change in the ordination cannot be observed. Especially, the ordination does not change related to the pesticide distribution: taxa linked to pesticide presence or absence does not change their behaviour in PCA outputs due to physical parameters.

Table 5.6. Data coordinates on axis 1 of the PCAs performed including and not including physical parameters.

PCA <u>including</u> physical parameters	**axis 1 (33%)**	**PCA <u>NOT including</u> physical parameters**	**axis 1 (20%)**
Temperature (2 cm depth)	28.9956		
Soil Density	17.9004		
Moisture	15.1565		
Prostigmata	5.9380	Katiannidae	12.6741
Katiannidae	4.3934	Isotomidae	10.7113
Isotomidae	4.1400	Prostigmata	10.2042
Coleoptera (larvae)	3.6442	Coleoptera (larvae)	9.4570
Coleoptera	3.1896	Coleoptera	5.9568
Diptera (larvae)	1.8596	Diptera (larvae)	5.7086
Entomobryidae	1.3170	Bourletiellidae	4.5666
Cryptostigmata	1.2367	Cryptostigmata	4.0375
Mesostigmata	0.9324	Entomobryidae	3.9019
copper oxychloride	0.3614	Sminthurididae	3.4412
Sminthuridae	0.0368	Mesostigmata	3.3159
copper sulphate	-0.0157	Sminthuridae	2.4355
Onychiuridae	-0.1110	Diplura	2.3209
Bourletiellidae	-0.1329	Onychiuridae	2.2865
Diplura	-0.2074	Astigmata	1.2926
Sminthurididae	-0.2302	Pauropoda	1.0429
sulfur	-0.5681	Symphyla	0.8913
Pauropoda	-0.9320	Araneae	0.8117
Symphyla	-1.2102	Hemiptera	0.7402
Araneae	-1.2950	Diptera	0.4409
cyprodinil	-1.3773	Hymenoptera	0.4023
glyphosate	-1.4787	Neanuridae	0.0688
Diptera	-1.5761	Lepidoptera (larvae)	-0.1099
Hemiptera	-1.5985	Thysanoptera	-0.1927
Astigmata	-1.6104	Protura	-0.3923
thiamethoxam	-1.6137	Psocoptera	-0.4969
Hymenoptera	-1.7490	Thysanoptera (nymphs)	-0.6790
Tullbergiidae	-1.7803	Formicidae	-0.7484
dimethomorph	-2.0134	Isopoda	-0.8085
fludioxonil	-2.0184	Hymenoptera (larvae)	-0.9396
chlorpyrifos	-2.0690	Chilopoda	-0.9443
Neanuridae	-2.2594	Tullbergiidae	-1.0381
Thysanoptera (nymphs)	-2.3807	Pseudoscorpionida	-1.1005
oxadiazon	-2.4181	Neelidae	-1.5857

Table 5.6. – continued.

PCA including physical parameters	**axis 1 (33%)**	**PCA NOT including physical parameters**	**axis 1 (20%)**
Protura	-2.5236	Hemiptera (larvae)	-1.7888
Thysanoptera	-2.5437	Formicidae (larvae)	-1.9225
Psocoptera	-2.5446	Diplopoda	-2.0740
iprovalicarb	-2.5934	carbendazim	-2.1398
Chilopoda	-2.6709	thiophanate-methyl	-2.3417
Lepidoptera (larvae)	-2.7082	folpet	-2.8440
carbendazim	-2.8081	mepanipyrim	-3.0141
Isopoda	-2.8524	mancozeb	-3.0706
mepanipyrim	-2.8807	chlorpyrifos	-3.7590
folpet	-2.9516	iprovalicarb	-3.9737
thiophanate-methyl	-2.9546	fludioxonil	-4.0417
Hymenoptera (larvae)	-3.0162	copper sulphate	-4.4128
Neelidae	-3.0290	copper oxychloride	-4.6860
mancozeb	-3.1265	dimethomorph	-5.2441
Diplopoda	-3.2014	cyprodinil	-5.3014
Hypogastruridae	-3.2598	thiamethoxam	-5.3194
Pseudoscorpionida	-3.2672	sulfur	-5.8713
Formicidae (larvae)	-3.5055	oxadiazon	-6.3762
Hemiptera (larvae)	-3.5502	glyphosate	-7.7643

Two considerations should be done. Physical parameters are driving forces in determining the community composition. Anyway, what we want to observe are the relative changes within the community and they seem to be related to chemical stressors more that to physical ones. In this work, however, a bias could be that physical parameters were measured only during samplings, thus they recorded the situation in that moment. In contrast, the structure of the community is dependent of the time before samplings. Anyway, differences of the physical parameter trends throughout the year among the three points never exceeded the variation ranges to which the different populations are adapted.
Keeping in mind these considerations, and the fact that physical parameters may play a role in the relative abundances of some groups, e.g. mites in June (section 5.3.1,figure 5.5), physical stressors seem not sufficient to explain the relative behaviour of different taxa in this field work.
Only PCA with community and chemicals is reported and discussed (figure 5.11).

Figure 5.11 shows the PCA output of the distribution of all input data (community and pesticides) on the first two axis. The explained variance is

relatively low (34%), but it is not surprising considering that it is a field work; anyway some trends can be observed.

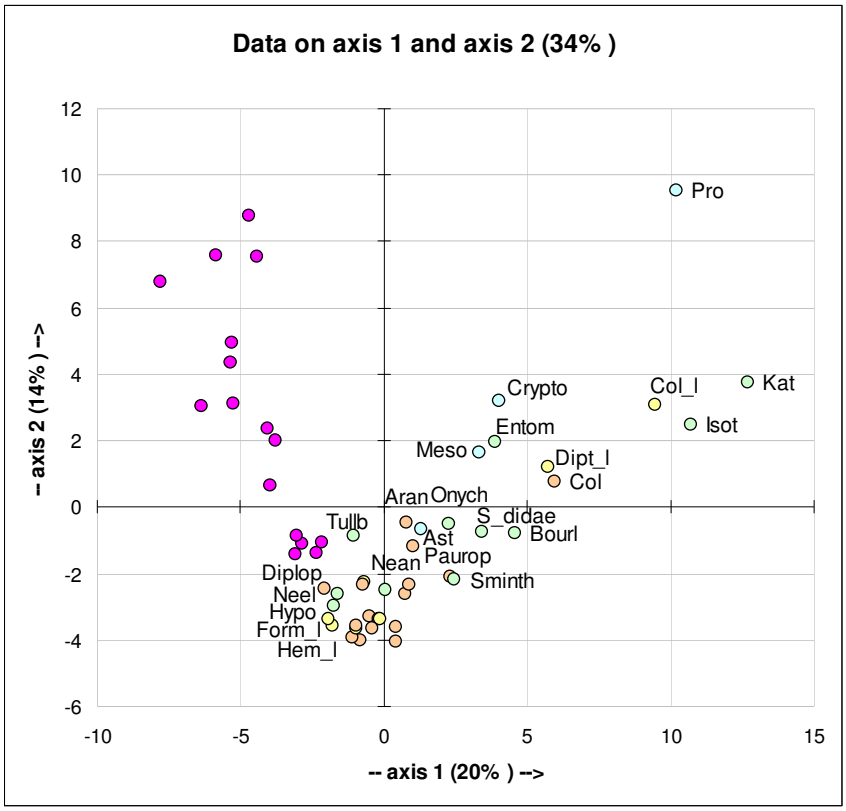

Figure 5.11. PCA output of the distribution of pesticides and community counts data on the first two axis. PPPs are reported in pink dots. Light blue dots: mites, green dots: springtails, orange dots: adult forms of other organisms, yellow dots: juveniles forms of other organisms. Not all the taxa names are reported, for sake of clarity of the figure, for others see abbreviations in Appendix A. (modified after Vaj *et al.*, 2010).

The driving force on axis 1 seems to be the presence of pesticide, having them negative values on axis 1 (left side of the graph) but being absent on the right side (positive values on axis 1). Distribution of the samples (not shown in figure) was guided by the PPP presence, having almost all A samples negative values on axis 1 and positive on axis 2, while B and C samples assumed positive values for both the axis, except few samples slightly below 0 on axis 2.

It could be hypothesized that also community distribution is related to pesticides one. Thus on axix 1 an inverse relationship between chemicals

and some organisms could be seen. Axis 1 can be seen as a "vulnerability axix" with higher values on the right side and lower on the left part of the graph.
Taxa that are in the opposite part of the graph respect to pesticides are those present in the samples with the lowest predicted concentrations of PPPs, because of the date (pre-applications) and the point (B or C). This is the case of prostigmatid mites, Katiannidae and Isotomidae (Collembola) and larvae of beetles. For the two springtails families and Coleoptera larvae this confirms what observed in the time trends and PRCs analyses (sections 5.3.1 and 5.3.2). The family of Katiannidae does not reach high numbers in this environment, as Isotomidae; anyway analysing the counts in each sample it is should be noted that, though the low numbers, they are never present inside the vineyard in post-application dates (JulyI, JulyII and September), while they are present outside and in A point in the other dates. Also numbers of Coleoptera larvae are low, but it seems that their distribution is more related to the annual cycle of species than to the pesticide presence, that overlaps PPP applications. Prostigmata distribution seems surprising, since it is not confirmed by previous analysis (e.g. PRC). They are the most abundant group of organisms found in almost all the samples, thus their position in the PCA output seem to be related to the high values found for some samples with low pesticide PECs, more than an absence inside the vineyard. Refined with this considerations, it could be concluded that Isotomidae and Katiannidae are taxa vulnerable to the chemical stress given by PPPs, because they are present when and where pesticide are considered to be in the lowest concentrations. Astigmatid mites do not show a negative relation with pesticide presence in the PCA output, as it happened in the PRCs. This can be due to the very low numbers that they reach compared to other taxa, especially other mites, that can bias their result in the PCA.
Cryptostigmata, Mesostigmata, Entomobryidae, Coleoptera and larvae of Diptera show a light tendency in the same direction.
On the opposite side of the graph, related to the pesticide presence, taxa that are likely to be present in samples inside the vineyard and in post-application dates are found. They are three springtails families (Hypogastruridae, Tullbergiidae and Neelidae), Formicidae (adults and larvae) Hemiptera larvae and Diplopoda. Formicidae and Hemiptera (larvae) can be considered rare in this environment, because they have been found sporadically. Ant larvae distribution are driven by one sample collected in A point in June09, with a very high ants community. Anyway, ants distribution is very clustered and they are social insects, thus their distribution is driven mainly by their behaviour. The other four taxa are also those with low B_k values in PRCs (section 5.3.2). They can be considered as less vulnerable organisms, because they are present when and where also pesticides are present.

5.4. Conclusions

In this field work the application of pesticides, especially the insecticide chlorpyrifos, seems to have an influence on microarthropod communities. In fact, the analysis of time trends in the community showed that some organisms are affected by chlorpyrifos applications, especially inside the vineyard. The chemical contamination seems to be more important than physical stress given by soil moisture or temperature fluctuations in the relative behaviour of the taxa within the community. Nevertheless, physical parameters are important for the overall behaviour of the whole community and sometimes can have an influence on relative abundances, as for astigmatid mites when insecticides are not present.
In September a recovery of the abundance of the community is evident outside the vineyard, but not inside. For the majority of taxa only at the beginning of the next spring a recovery can be observed within the field. A general recovery one year after the beginning of the investigation is recorded, except for springtails.
Multivariate statistical analyses allowed the identification of the taxa more affected by the PPP presence and those favoured by the chemical stressor. Two springtail families, Isotomidae and Katiannidae, and astigmatid mites seem to be the taxa more affected by pesticides, thus they are considered to be more vulnerable to their presence. On the other hand, Hypogastruridae, Tullbergiidae and Neelidae (Collembola), pauropods and diplopods seem to be favoured by pesticide applications, and this could be a result of both an indirect effect of the absence of other taxa and a minor vulnerability of them to this kind of stressor. Other taxa showed similar behaviours as these two groups, but it is considered to be due mainly to characteristics not necessarily related with the pesticide presence, as emergence of flying organisms (e.g. some Coleoptera) and social behaviour of ants.
Vulnerability of taxa has been assessed with rough methods and seem to play a role in the behaviour of the community throughout the year in relation to chemical stress given by the pesticide presence. Further analyses were thus performed with most suitable conceptual tools and are presented in chapters VII an VIII.

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References

Angelini P, Fenoglio S, Isaia M, Jacomini C, Migliorini M, Morisi A, 2002. *Tecniche di biomonitoraggio della qualità del suolo*. ARPA Piemonte, Torino, Italy, 106 pp. In Italian.

Bachelier G, 1986. *La vie animale dans le sol.* O.R.S.T.O.M., Paris, France, pp. 171-196. In French.

Barmaz S, 2009. *Plant protection product risk assessment: distribution and experimental validation in terrestrial ecosystems.* PhD thesis, Doctorate in Environmental Sciences (XXII cycle), Faculty of Mathematical, Physical and Natural Sciences, University of Milano – Bicocca, Milano, Italy, 113 pp. http://hdl.handle.net/10281/7503.

Bellinger PF, Christiansen KA, Janssens F, 1996-2010. Checklist of the Collembola of the World. http://www.collembola.org (accessed from October 2008 to April 2010).

Bolt GH and Bruggenwert MGM (Eds.), 1976. *Soil chemistry, A: Basic elements*. Developments in Soil Science 5A. Elsevier, Amsterdam, The Netherlands, 281 pp.

Butcher JW, Snider R, Snider RJ, 1971. Bioecology of edaphic Collembola and Acarina. *Annu Rev Entomol* 16:249-288.

Chinery M, Pandolfi M (Ed), 1998. *Guida degli insetti d'Europa – atlante illustrato a colori*. Franco Muzzio Editore, Padova, Italy, 431 pp. In Italian.

Choi WI, Ryoo MI, Kim J-G, 2002. Biology of *Paronychiurus kimi* (Collembola: Onychiuridae) under the influence of temperature, humidity and nutrition. *Pedobiologia* 46:548-557.

Codurri M, Truzzi A, Bertonazzi MC, 2005. *Microartropodi del terreno – manuale da campo per il riconoscimento dei microartropodi del terreno come indicatori della qualità biologica del suolo (metodo QBS-ar)*. Consorzio del parco naturale dell'Oglio sud, Calvatone (CR), Italy, 104 pp. In Italian.

FOOTPRINT website, 2006. The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.eu-footprint.org/ppdb.html (accessed in June 2009).

Frampton GK, 2000. Recovery responses of soil surface Collembola after spatial and temporal changes in long-term regimes of pesticide use. *Pedobiologia* 44: 489-501.

Ganzelmeier H, Rautmann D, Spangenberg R, Streloke M, Hermann M, Wenzelburger HJ, Walter HF, 1995. *Studies on the spray drift of plant protection products. "Mitteilungen aus der Biologischen Bundesanstalt für*

Land- und Forstwirtschaft". No 305, Blackwell Wissenschafts-Verlag, Berlin, Germany, 111 pp.
Hopkin SP, 2007. *A key to the Collembola (springtails) of Britain and Ireland*, FSC, Shrewsbury, UK. 245 pp.
Huhta V and Hänninen S-M, 2001. Effects of temperature and moisture fluctuations on an experimental soil micro arthropod community. *Pedobiologia* 45:279-286.
ISO (International Organization for Standardization), 2006. ISO/FDIS 23611-2 *Soil quality- Sampling of soil invertebrates – Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina).* International Organization for Standardization, Geneva, Switzerland, 12 pp.
Jackson JE, 1991. *A user's guide to principal components*. Wiley, New York (NY), USA, 592 pp.
Jolliffe IT, 1986. *Principal Component Analysis.* Springer Series in Statistics, Springer, New York (NY), USA, 487 pp.
Koolhas JE, Van Gestel CAM, Römbke J, Soares AMVM, Jones SE, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on microarthropod communities. *Ecotoxicology* 13:75-88.
Leistra M, 2005. *Estimating input data for computations on the volatilisation of pesticides from plant canopy and competing processes*. Alterra-rapport 1256, Alterra, Wageningen, The Netherlands, 80 pp.
Liess M and von der Ohe PC, 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ Toxicol Chem* 24: 954-965.
Malmström A, 2008. Temperature tolerance in soil microarthropods: simulation of forest-fire heating in the laboratory. *Pedobiologia* 51:419-426.
Parisi V, 2001. La qualità biologica del suolo. Un metodo basato sui microartropodi. *Acta naturalia de "L'Ateneo Parmense"* 37: 97-106. In Italian.
Simpson EH, 1949. Measurements of diversity. *Nature* 163: 688.
Shannon CE and Weaver W, 1963. *The mathematical theory of communication*. The University of Illinois Press, Urbana (IL), USA, 117 pp.
Soil Survey Division Staff, 1993. *Soil survey manual*. Soil Conservation Service. U.S. Department of Agriculture (USDA), Handbook 18, Whashington (DC) USA, 437 pp.
Todeschini R, 1998. *Introduzione alla chemiometria.* EdiSES, Napoli, Italy, 321 pp. In Italian.
Tomlin, CDS (Ed). *The pesticide manual: a world compendium (13th ed.)*. British Crop Protection Council, Farnham, UK, 1606 pp.
Vaj C, Van Gestel CAM, Vighi M, 2010a. Year-round monitoring of soil microarthropod communities under pesticide application and other stressors in a real case. Presented to SETAC Europe XX meeting "Science and technology for environmental protection", 23rd – 27th May 2010, Seville, Spain.

Van den Brink PJ and Ter Braak CJF, 1998. Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. *Aquat Ecol* 32: 163-178.
Van den Brink PJ and Ter Braak CJF, 1999. Principal Response Curves: analysis of time-dependent multivariate responses of biological community to stress. *Environ Toxicol Chem* 18: 138-148.
Van den Brink PJ, den Besten PJ, bij de Vaate A, Ter Braak CJF, 2009. Principal response curves technique for the analysis of multivariate biomonitoring time series. *Environ Monit Assess* 152: 271-281.
Verro R, Finizio A, Otto S, Vighi M, 2009. Predicting pesticide environmental risk in intensive agricultural areas. I: screening level risk assessment of individual chemicals in surface waters. *Environ Sci Technol* 43: 522-529.
Vighi M, Finizio A, Villa S, 2006. The evolution of the environmental quality concept: from the US EPA Red Book to the European Water Framework Directive. *Environ Sci & Pollut Res* 13: 9-14.
Wiles JA and Frampton GK, 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. *Pestic Sci* 47: 273-285.

CHAPTER VI

Attempt to identify indirect effects of the application of pesticides on the structure of the food web in microarthropod community

Abstract

The application of Plant Protection Products (PPP) can affect non-target species, both for the direct adverse effects on them or for the indirect one, due to the effect of the pesticide on another organism. In food webs the indirect effect could be linked to the depletion of a food sources by the pesticide. A microarthropod community was sampled in a vineyard in Northern Italy where pesticides are used. In particular, to evaluate indirect effects, three samples were examined: two after the application of herbicides and fungicides but before application of the insecticide chlorpyrifos, and the third after chlorpyrifos application. The sampled organisms were divided according to their food preferences into six groups. Time trend of each group within the vineyard was observed compared to a control point 10 m far from the field. Some differences in the trends could be observed among the groups. Overall, the direct effect of chlorpyrifos seemed to be more important the indirect ones.

Keywords: indirect effects, food web, pesticides, microarthropods.

6.1. Introduction

The complexity of ecosystems is one of the major critical points for a proper prediction of ecotoxicological effects in the field. Linkages within and between populations are capable to influence the overall functioning of an ecosystem and its responses to stressors. Beside the direct effect of a perturbation on a part of the ecosystem, indirect effects can take place to other components. An example could be the prey-predator relationship: if preys decrease due to a stressor, also predator would decrease for the depletion of food source, if they cannot find a suitable alternative one. Indirect effects on the food relations among the components of an ecosystem could be due to the pesticide application in agroecosystems. These products have been developed to kill the target organisms (fungi, plants or insects), but may give adverse effects also on non-target species. The effects can be direct on the species, or indirect, due to the effect of the pesticide on another organism, for example the effect of a fungicide on fungi eaten by fungivorous animals.

Focusing on soil organisms, some studies revealed indirect effects of pesticide use on microarthropods. Some example are shown.

Frampton (1997) studied the behaviour of some springtail species in wheat fields under the applications of insecticides, herbicides and fungicides. He found *Lepidocyrtus* spp. (both *L. cyaneus* Tullb. and *L. violaceus* Lubb., family Entomobryidae) to increase in a field with low application regime and addressed it to an indirect effect of fungicides and/or herbicides on microflora, microfauna and weed populations or subtle variations in soil properties.

In a field work using the fungicide carbendazim, Koolhas *et al.* (2004) found astigmatid mites significantly decreasing with the two highest dosages, while Prostigmata, Cryptostigmata, and Mesostigmata showed no effects. They explained the reduction of Astigmata by the reduction of their food source by the fungicide, concluding that it was an indirect rather than a direct effect of carbendazim treatment.

Endlweber *et al.* (2006) observed effects of two insecticides, chlorpyrifos and dimethoate, used respectively on soil and on vegetation, on collembolan community in an early set-aside arable field. They observed that, in addition to direct toxicity, both insecticides may have affected springtails density indirectly. Indeed, some predators not affected by insecticide applications might have been advantaged by a release from competition and reduced collembolan densities. On the other hand, densities of competitors for food sources might have increased and caused a further decline in springtails numbers.

Investigating a broader soil community, Rutgers (2008) observed plausible direct or indirect effects (e.g. by a change in the nutritional status of the soil) on nematodes by metals.

In this chapter an attempt to identify indirect effects of pesticide applications on a real microarthropod community was performed. The community was the same presented in chapter V, thus the work was strictly linked to a field situation and not based on food webs drawn by other authors, as e.g. Hunt *et al.* (1987) in a shortgrass prairie or Berg *et al.* (2001) in a Scots pine forest soil.

6.2. Materials and methods

6.2.1. Community and diet data used in the analysis

The analysis of indirect effects on food webs was referred to the field situation described in chapter V. A field campaign was performed, starting in June 2008 until June 2009, in a vineyard in Veneto region, Northern Italy, where pesticides are used. In particular, to evaluate indirect effects, three samples were examined: two after the application of herbicides and fungicides but before application of the insecticide chlorpyrifos, and the third after chlorpyrifos application. All the information about the active ingredients used was obtained and the exposure of the community was assessed with traditional ecotoxicological tools (chapter III, V) (figure 6.1).
Microarthropod samples were collected in replicates in three points in an exposure gradient, as shown in figure 6.2, using a split corer of 10 cm diameter and up to a depth of 10 cm. All the sampled organisms were identified to the order level, except ants and springtails, for which family level was reached, and mites, that were divided into four major groups. Details on sampling and identification methods are reported in chapter V, sections 5.2.1 and 5.2.2.

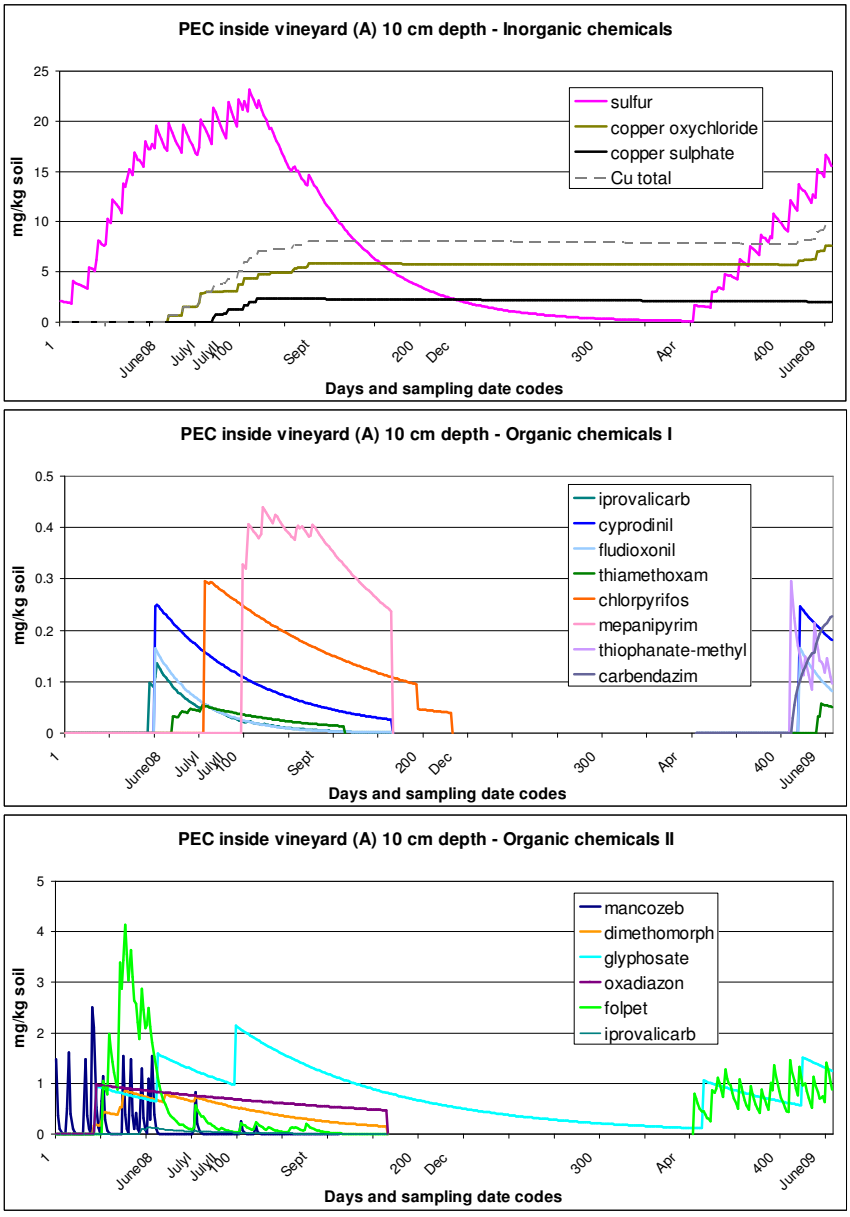

Figure 6.1. PEC (mg/kg soil) in soil (depth 10 cm) within the vineyard (divided in inorganic and organic chemicals for sake of clarity). On x-axis sampling date codes are reported (June08: sampling on 23rd – 24th June 2008; JulyI: sampling on 15th July 2008; JulyII: sampling on 29th July 2008; Sept: on 16th September 2008; Dec: sampling on 2nd – 3rd December 2008; Apr: sampling on 8th April 2009; June09: sampling on 23rd – 24th June 2009). Days are reported as numbers.

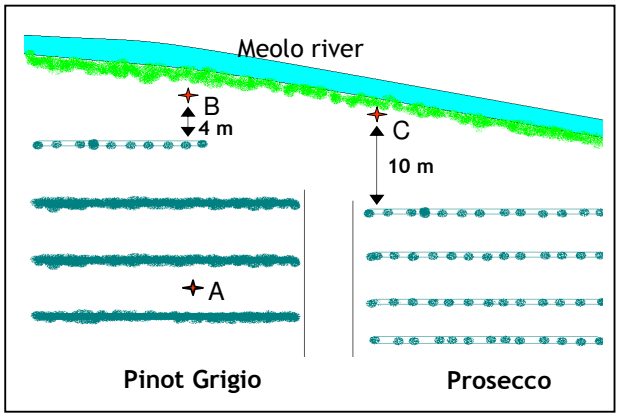

Figure 6.2. Field site scheme (modified after Vaj *et al.*, 2010).

Information on food preferences were collected from literature. Six groups of food were identified:

- bacteria;
- fungi;
- dead organisms, organic matter and excrements, grouped together as structured detritus of animal origin, "sapr/necr";
- amorphous detritus, high degree of decomposition;
- plant materials (leaves, roots, mycorrhizae, algae, structured vegetal detritus);
- animals, both for predators or parasites second consumers.

The three categories comprising detritus were identified focusing on the origin of the detritus, because it is assumed that a plant protection product (PPP) would act on the organism from which it derives, in different ways if it is an animal or a plant.

For each taxon a value ranging from 0 to 1 according to the affinity with the food group was given based on literature information (table 6.1). For springtails differences cold be seen only for the family Bourletiellidae; for the others information found for the whole order Collembola was used and addressed to the different families.

Table 6.1. Affinity of each taxon to the six category of type of food.

TAXON	type of food					
	bacteria	**fungi**	**sapr/necr**	**detritus**	**plant materials**	**animals**
Isopoda		0.1[(a)]	0.1[(a)]	0.1[(a)]	0,7[(a,b)]	
Pseudoscorpionida						1[(a,c)]
Araneae						1[(a)]
Astigmata		0.111[(d)]		0.167[(d)]	0.389[(d)]	0.333[(d)]
Prostigmata		0.188[(d)]			0.188[(d)]	0.625[(d)]
Cryptostigmata	0.056[(d)]	0.056[(d)]	0.333[(d)]	0.167[(d)]	0.389[(d)]	
Mesostigmata		0.125[(d)]			0.25[(d)]	0.625[(d)]
Hemiptera					1[(e)]	
Hemiptera (larvae)					1[(f)]	
Psocoptera		0.321[(e)]		0.214[(e)]	0.464[(e)]	
Thysanoptera		0.05[(e)]			0.9[(e)]	0.05[(e)]
Thysanoptera (nymphs)		0.05[(f)]			0.9[(f)]	0.05[(f)]
Coleoptera		0.033[(e)]	0.268[(e)]	0.021[(e)]	0.420[(b,e)]	0.259[(e)]
Coleoptera (larve)		0.012[(e)]	0.151[(e)]	0.012[(e)]	0.420[(b,e)]	0.404[(e)]
Lepidoptera (larvae)		0.053[(e)]			0.947[(e)]	
Hymenoptera					0.133[(e)]	0.867[(e)]
Hymenoptera (larvae)		0.028[(e)]			0.194[(e)]	0.778[(e)]
Formicidae		0.125[(a)]			0.5[(e)]	0.375[(e)]
Formicidae (pupae)					0.333[(e)]	0.667[(e)]
Diptera			0.5[(e)]		0.25[(e)]	0.25[(e)]
Diptera (larve)		0.125[(a)]	0.375[(e)]		0.125[(e)]	0.375[(a,e)]
Protura		0.333[(b)]			0.333[(b,e)]	0.333[(a)]
Diplura		0.042[(a)]	0.25[(g)]	0.292[(b)]	0.042[(a)]	0.375[(a,g)]
Hypogastruridae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Neanuridae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Onychiuridae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Tullbergiidae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Isotomidae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Entomobryidae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Bourletiellidae		0.2[(h)]	0.2[(h)]		0.6[(h)]	
Katiannidae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Sminthuridae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Sminthurididae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Neelidae		0.231[(a)]		0.231[(a)]	0.462[(a)]	0.077[(a)]
Diplopoda					1[(a)]	
Chilopoda					0.2[(a)]	0.8[(a)]
Pauropoda	0.143[(a)]	0.393[(a,g)]			0.143[(a)]	0.321[(a,g)]
Symphila	0.125[(a)]	0.125[(a)]			0.5[(a,g)]	0.25[(g)]

(a) Codurri *et al.*, 2005; (b) Angelini *et al.*, 2002; (c) Barnes, 1985; (d) Evans, 1992; (e) Chinery, 1998; (f) Berg and Van Gestel, personal communication; (g) Coleman, 2003; (h) Bretfeld, 1999.

6.2.2. Calculation of trends

The sampled organisms were counted for each sampling date and point and averages values of the replicates were calculated.
The results were multiplied by the values in table 6.1, for each taxon, for obtaining the proportion of the food preference in the community in a particular date and point. For each food group the results found for each taxon were summed together for each point and date, for assessing a time trend.
Being the point C the farthest from the vineyard (10 m), it was taken as control. Thus, all the values were normalised according to C results (eq. 6.1):

$$x'_{i,j} = x_{i,j} / x_{i,j,C} \qquad \text{(Eq. 6.1)}$$

where:
$x'_{i,j}$ is the new value assumed for i-th food category by a sampling point, in the j-th sampling date;
$x_{i,j}$ is the original value assumed for i-th food category by a sampling point, in the j-th sampling date;
$x_{i,j,c}$ is the value assumed for i-th food category by sampling point C, in the j-th sampling date.

Normalising the average values to a control point make it impossible to perform an ANOVA on it. Significativeness of differences were thus performed on the averages values before normalisation, using Minitab 15.1.30.0 (One-way ANOVA with a code taking into account both point and date as factor).

6.3. Results and discussion

Following the procedures described in section 6.2, time trends of the food groups were constructed and are presented from figure 6.3 to figure 6.8. The community was normalised to the control level, thus only deviations from this level are reported. For sampling dates code see figure 6.1.

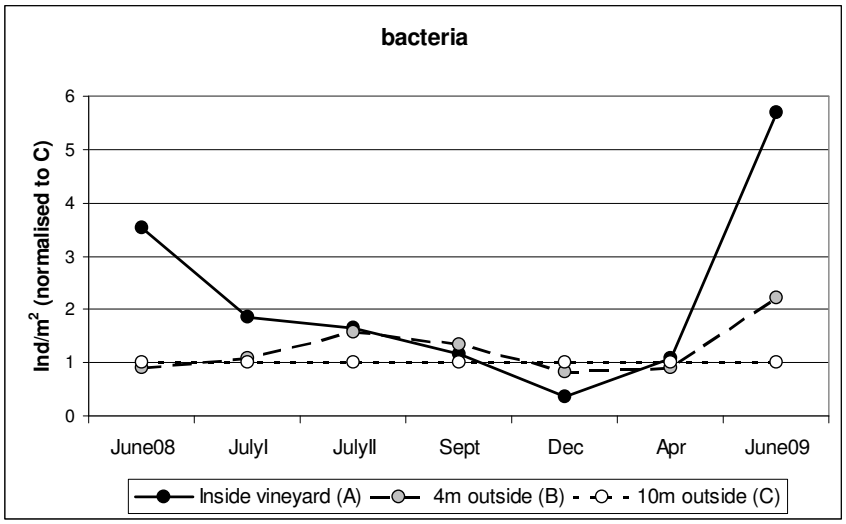

Figure 6.3. Time trend for the organisms feeding on bacteria. Significant differences calculated before normalisation to C point were found in A point in the couple of dates Sept-Dec (P<0.01).

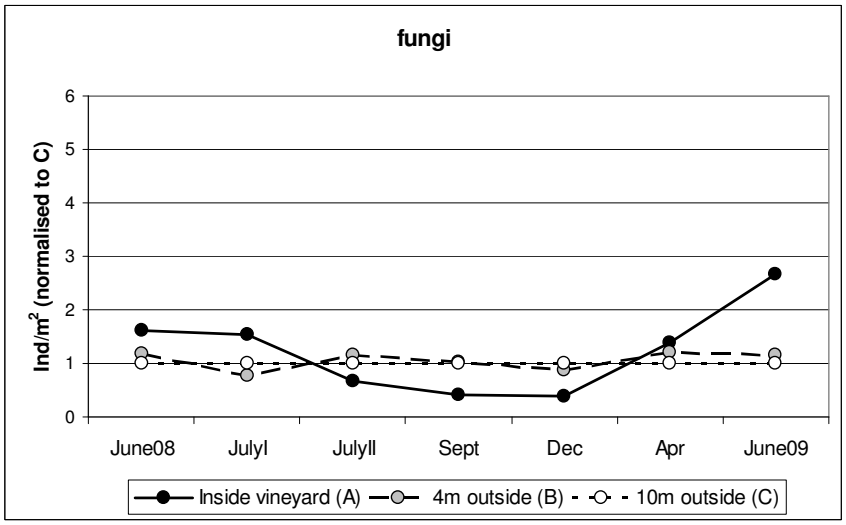

Figure 6.4. Time trend for the organisms feeding on fungi. Significant differences calculated before normalisation to C point were found in Sept A-B (P<0.01) and A-C (P<0.0.5), in Dec A-B (P<0.01) and A-C (P<0.05), in A point in the couples of dates JulyI-JulyII (P<0.05) and Sept-Dec (P<0.05), in B point for the couple JulyII-Sept (P<0.01).

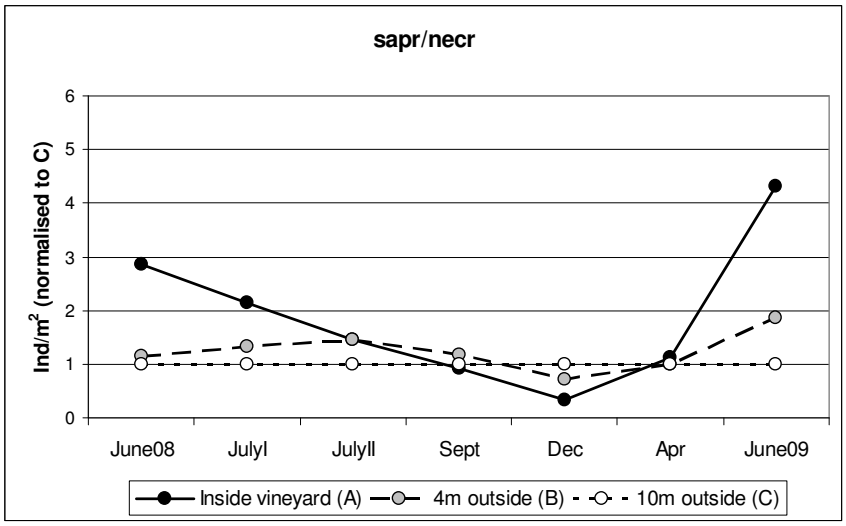

Figure 6.5. Time trend for the organisms feeding on dead organisms, organic matter and excrement, grouped as structured detritus of animal origin. Significant differences calculated before normalisation to C point were found in A point in the couple of dates Sept-Dec ($P<0.01$).

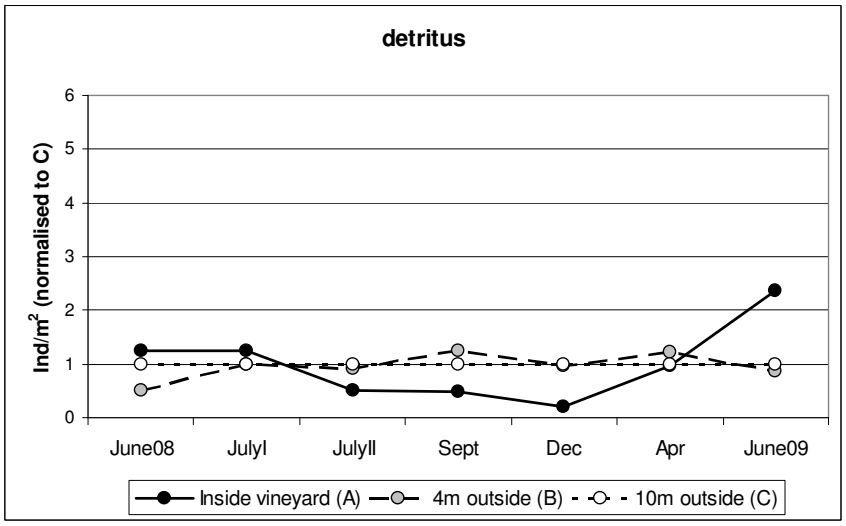

Figure 6.6. Time trend for the organisms feeding on amorphous detritus. Significant differences calculated before normalisation to C point were found in Sept A-B ($P<0.01$), Dec A-B ($P<0.01$), in A point between for the couples of dates JulyI-JulyII ($P<0.01$), Sept-Dec ($P<0.05$) and Dec-Apr ($P<0.01$) and in B point for the couple JulyII-Sept ($P<0.01$).

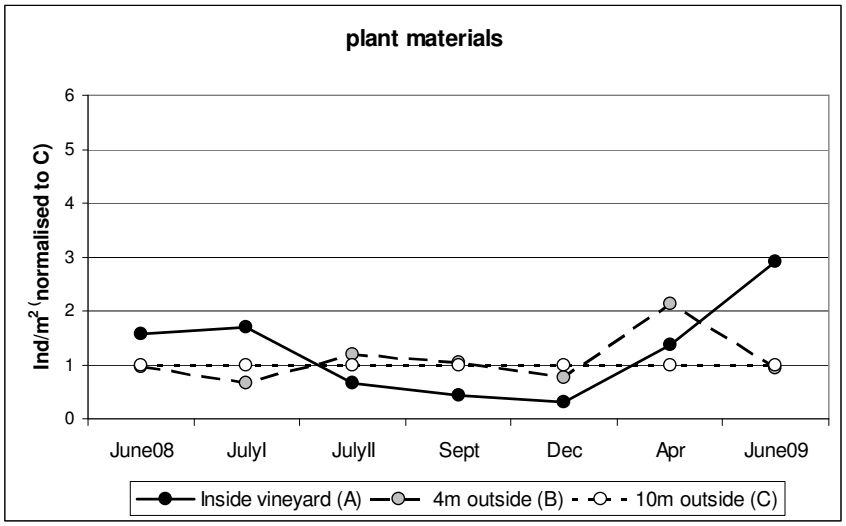

Figure 6.7. Time trend for the organisms feeding on plant materials. Significant differences calculated before normalisation to C point were found in Sept A-B ($P<0.01$) and A-C ($P<0.05$), in Dec A-B ($P<0.01$) and A-C ($P<0.05$), in A point between the couples of dates Sept-Dec ($P<0.05$) and Dec-Apr ($P<0.01$) and in B point for JulyII-Sept ($P<0.01$).

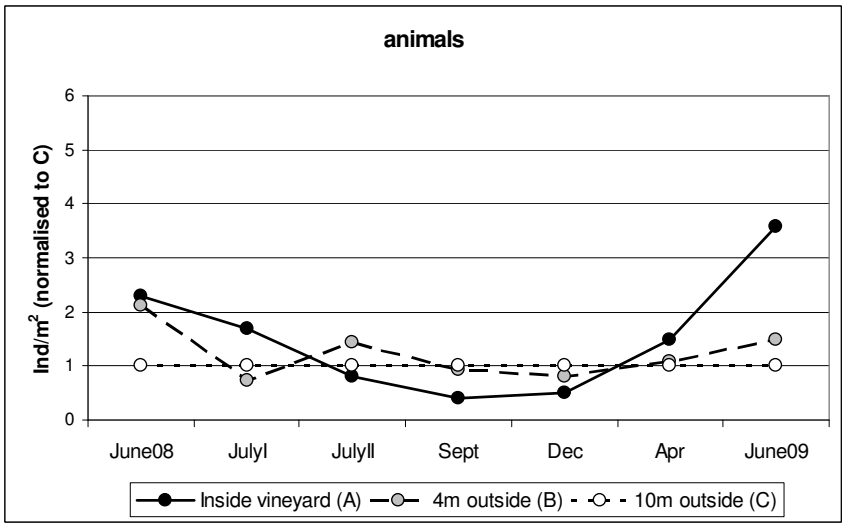

Figure 6.8. Time trend for the organisms feeding on animals (as predators or parasites). Significant differences calculated before normalisation to C point were found in Sept A-B ($P<0.05$) and A-C ($P<0.05$) and in June09 A-C ($P<0.05$).

The hypothesis was that before the use of chlorpyrifos, applied between JulyI and JulyII dates, there could be indirect effects on the abundance of organisms due to the elimination of food sources, while after chlorpyrifos application the direct effect of the insecticide should become visible. Chlorpyrifos, indeed, is an insecticide, that have an effect also on non-target species and can cause adverse effects to a community, as shown in chapter V.
Focusing on the first three sampling dates, some differences in trend could be observed. From June08 to JulyI fungicides, herbicides and the insecticide thiamethoxam were applied. As highlighted in chapter V, the community was just slightly affected after the application of these pesticides, and strongly decreased in abundance after chlorpyrifos application. Thus in the first two dates a direct effect due to plant protection products is considered as negligible, and indirect effect could be seen. Between JulyI and JulyII chlorpyrifos was applied and the direct effect could be observed. The trends were analysed for these three dates, because are those giving indications of indirect or indirect effects of PPPs

The analysed trends are those inside the vineyard, that is the point where pesticides are directly applied. Statistically significant differences from the control for B point were never found and it could be considered as intermediate between A and C. ANOVAs calculated for A point showed seldom statistically significant differences, anyway some indications of trends could be seen and are discussed.
For all the six groups starting levels inside the vineyard seems to be higher than the control (only as indication, because P was not lower than 0.05). For all them, numbers increased from the beginning of the next spring, showing a possible recovery to the starting levels. Also in this case this is an indication, needing a statistical confirmation.
It seemed that bacteriophages show a sharp decrease in the first period, but no differences were detected after chlorpyrifos application. It seems that for this group an indirect effect due to the depletion of the food source can be visible. Most soil bacteria, indeed, live close to plant roots. Herbicide application, thus, may have altered rhyzosphere and thus this food source. Unfortunately the statistics does not support this hypothesis, and it could be proposed only as an indication of trend, to be confirmed.
On the other hand, fungivores, detritivores and herbivores were almost constant from June08 to JulyI and decreased in the chlorpyrifos post application date (for fungivores and detritivores $P<0.05$ and $P<0.01$ respectively, for herbivores just indications). This is surprising, because an indirect effect of the food depletion was expected, at least for organisms feeding on fungi and on plant materials. The explanation could be related to the homeostatic processes within the food web. Indeed, if the food web has enough redundancy, depletion of one food source is balanced by the

consumption of another. Anyway, for fungivores and detritivores direct effect of chlorpyrifos is evident, while for herbivores a statistical confirmation is needed.
Saprophages, necrophages and secondary consumers seemed to decrease in all the analysed period. Focusing on the first dates, this could be the result of the decreasing of food source (e.g. bacteriophages or taxa not included in the community, as nematodes) as indirect effect. Also in this case, a direct effect of chlorpyrifos seems to exists, although a statistical confirmation is needed.
The direct effect of the insecticide is evident for fungivores and detritivores, being more important than indirect ones. Indications of similar behaviour in saprophages/necrophages (P=0.06), secondary consumers (P=0.058) and herbivores seem to exist. An important point regarding indirect effects related on diet is the homeostasis of the food web, thus if a source is depleted, others can be used. Moreover, as shown in table 6.1 only seldom a taxon feed only on one food source, increasing the stability of the food web.

As all the works based on field campaign, the presented results are affected by the complexity of the ecosystem. Soil, in particular, shows high variations even in the replicates of the same samples. Increasing the sample size could reduce the uncertainties in detecting trends and differences. Anyway, the sample size is often the best compromise costs/benefits.

6.4. Conclusions

An analysis of indirect effects of pesticides applied in a vineyard was performed, related to a field campaign of counting and identifying microarthropods. Two periods of investigation were interesting: before chlorpyrifos application, with other products used in field, and post chlorpyrifos application.
Although not always statistically confirmed, different trends could be observed in the community, according to the food preferences of the taxa, and were related to indirect or direct effect of products. For bacteriophages indications of an indirect effect due to the depletion of food sources could be seen, but not for fungivores, herbivores and detritivores. Saprophages, necrophages and secondarily consumers seemed to be affected by both the effects: the fist, indirect, due to a possible decreasing of food source and preys (not only those included in the community, as e.g. nematodes), and the second, direct, given by the insecticide.
The ratio between indirect and direct effects was investigated and for the majority of the taxa direct effects seemed to be more important than indirect ones.
Being this work based on a natural community, bias due to the complexity of the field conditions cannot be completely eliminated. Moreover, statistics not

always supported the observations and, ideally, confirmations are needed. The presented work, thus, does not aims to give general indications, but only a description of a field situation from the direct/indirect effect point of view.

Acknowledgments

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References

Angelini P, Fenoglio S, Isaia M, Jacomini C, Migliorini M, Morisi A, 2002. *Tecniche di biomonitoraggio della qualità del suolo*. ARPA Piemonte, Torino, Italy, 106 pp. In Italian.

Barnes RD, Milani R (Ed.), 1985. *Zoologia: gli invertebrati*. Piccin Nuova Libraria, Padova, Italy, 1237 pp. In Italian.

Berg M, de Ruiter P, Didden W, Janssen M, Schouten T, Verhoef H, 2001. Community food web, decomposition and nitrogen mineralisation in a stratified Scots pine forest soil. *Oikos* 94:130-142.

Bretfeld M, 1999. *Synopses on Palearctic Collembola Volume 2 SYMPHIPLEONA*. Staatliches Museum für naturkunde Görlitz, Görlitz, Germany, 318pp.

Chinery M, Pandolfi M (Ed), 1998. *Guida degli insetti d'Europa – atlante illustrato a colori*. Franco Muzzio Editore, Padova, Italy, 431 pp. In Italian.

Codurri M, Truzzi A, Bertonazzi MC, 2005. *Microartropodi del terreno – manuale da campo per il riconoscimento dei microartropodi del terreno come indicatori della qualità biologica del suolo (metodo QBS-ar)*. Consorzio del parco naturale dell'Oglio sud, Calvatone (CR), Italy, 104 pp. In Italian.

Coleman DC, 2003. Secondary production: activities of heterotrophic organisms – the soil fauna. In: Coleman DC, Crossley jr. DA (Eds.), *Fundamentals of soil ecology*, Academic Press, London, UK, 195 pp.

Endlweber K, Schädler M, Scheu S, 2006. Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field. *Appl Soil Ecol* 31: 136-146.

Evans GO, 1992. *Principles of acarology*. C.A.B. International, Cambridge, UK, 563 pp.

Frampton G, 1997. The potential of Collembola as indicators if pesticide usage: evidence and methods from the UK arable ecosystem. *Pedobiologia* 41: 179-184.

Hunt HW, Coleman DC, Ingham ER, Elliott RE, Moore JC, Rose SL, Reid CPP, Morley CR, 1987. The detrital food web in a shortgrass prairie. *Biol Fertil Soils* 3: 57-68.

Koolhas JE, Van Gestel CAM, Römbke J, Soares AMVM, Jones SE, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on microarthropod communities. *Ecotoxicology* 13:75-88.

Rutgers M, 2008. Field effects of pollutants at the community level – experimental challenges and significance of community shifts for ecosystem functioning. *Sci Total Environ* 406: 469-478.

Vaj C, Van Gestel CAM, Vighi M, 2010. Year-round monitoring of soil microarthropod communities under pesticide application and other stressors in a real case. Presented to SETAC Europe XX meeting "Science and technology for environmental protection", 23rd – 27th May 2010, Seville, Spain.

CHAPTER VII

Trait-based approach applied on microarthropod communities affected by pesticide applications

Abstract

The commonly used tools to assess ecotoxicological risk are lacking ecological realism, because they cannot predict the real consequences for natural communities. Recently the trait-based approach has been introduced in ecotoxicology. It is based on the hypothesis that the sensitivity of an organism to a stressor can be predicted from its biological characteristics.
A microarthropod community was sampled in a vineyard in Northern Italy under the application of pesticides, in a year long monitoring campaign. Traits of the sampled organisms were identified and quantified in a matrix. The results of the abundances of the sampled community were intersected with the quantified traits. The procedure allowed the identification of driving forces in the vulnerability of the organisms, as vertical stratification, instars, strategies against adverse conditions, number of generations per year, respiration type and presence of cuticle. More and less vulnerable taxa were identified according to these traits. The results are compared with those found in the trend analysis reported in chapter V.

Keywords: microarthropods, traits, vulnerability, pesticides.

7.1. Introduction

Traditional methods used for the ecotoxicological risk assessment for pesticides reveal a low ecological realism, because they does not take into account interactions among populations, indirect effects or, generally, the homeostatic capabilities of natural communities (Vighi *et al.*, 2006). In the previous chapters, for example, the results of a risk assessment for the soil environment together with an investigation on the natural community have been shown. Although the effect of the most toxic insecticide applied in the field site is clear in the natural community behaviour, a series of indirect effects given by the absence of a particular taxon and the lowest vulnerability of others have been identified. It is not possible to detect this kind of effects in a traditional risk assessment framework and, ideally, field works are needed. Considering the impossibility to establish experimental studies on all the communities and their populations, the need to produce suitable tools is felt. One of these new tools in ecotoxicology is the trait-based approach. The method uses biological characteristics of organisms to predict their sensitivity to a toxic substance. The hypothesis is that the sensitivity of an organism to a stressor is a function of their biological characteristics and can be predicted by morphological, life history, physiological and ecological traits that describe their physical attributes, ecological niche and functional role in ecosystem (Baird and Van den Brink, 2007; Baird *et al.*, 2008). This approach arise from the necessity to assess toxic effects on all the species at risk in an ecosystem, also those for which ecotoxicological data are not available in the literature. It is thus possible to overcome a risk assessment based on the few indicator species that are used traditionally. One of the major advantages is that the taxonomical unit is not the unit of the analysis anymore, but the focus is moved on the attributes linked to the sensitivity of a species to a stress. Indeed, different life stages of the same species can have different ecological functions and roles within the trophic web, while different species may have similar roles in the ecosystem, as the principle of the function redundancy shows (Baird *et al.*, 2008). For example, the biological characteristics and the ecological role of insect larvae or juveniles are generally very different compared to those of the adults. Thus the community can be described as a list of traits of the species rather than a taxonomic list of species. As important traits we intend those biological characteristics that can explain a particular behaviour of an organism.

A trait based ecological risk assessment (TERA) has been performed by Baird and van den Brink (2007) showing that four species traits explained 71% of the variability in sensitivity to toxicants within a group of 12 freshwater species exposed to 15 different substances. Liess *et al.* (2005; 2008) developed a tool to identify species at risk (SPEAR) based on species traits. Linking traits to sensitivity and to risk seems a promising approach, a

way that is likely to be followed in the modern ecotoxicology. As almost all the concepts developed in this topic, the starting environment is the freshwater compartment, for which, traditionally, more information is available. For example, as highlighted in chapter III, even simple ecotoxicological endpoints are missing for the soil microarthropod community.
Even if TERA is a recent concept, the use of biological traits for better analysing the behaviour of organisms has been yet applied in the past years. Tranvik *et al*. (1993) studied the behaviour of two collembolan species (*Onychiurus armatus* and *Isotoma notabilis*) under metal stress in relation with some traits as body length, number of eggs laid, average length of life, type of reproduction and vertical stratification, and their evolution under stress. Siepel (1995) described modifications in microarthropod communities under different kind of stressors grouping organisms according to their life-history tactics. Two recent studies on springtails have investigated traits in relation to food quality (Jørgensen *et al.*, 2008) and habitat preference and dispersal ability (Auclerc *et al.*, 2009). Makkonen *et al.* (2011) highlighted the importance of a trait-based approach and its major sensitivity in revealing community responses following climate changes in sub-arctic springtails, concluding that drought-tolerant, large-sized and epiedaphic species survived better under climate manipulation.
Also the recent ecological vulnerability analysis, that will be discussed in chapter VIII, uses traits to assess the vulnerability of an organism to a stressor. Briefly, vulnerability is defined as a function of three components: sensitivity, susceptibility to exposure and recovery potential after a stress and is a very stress-specific concept (De Lange *et al.*, 2009). Each species attribute would play a role in defining one of the components of the vulnerability.

In this chapter a trait based approach has been applied to the microarthropod community presented in chapter V and the behaviour and vulnerability of different taxa were explained on the basis of their attributes.

7.2. Materials and methods

7.2.1. Microarthropod community used in the analysis

The community used in this work is the same presented in chapter V, sampled in a vineyard in Veneto (Northern Italy) under the application of plant protection products (PPP). Sampling points were located in a decreasing exposure gradient, from inside the field (A point) to outside (4 and 10 m far away, B and C respectively), as shown in figure 7.1. Sampling started in June 2008 and lasted all the year, until June 2009, according to the

insecticide applications during the productive season (figure 7.2). Samples were collected in replicates using a split corer of 10 cm diameter, up to a 10 cm depth, and organisms were identified to taxa level. The exposure of the organisms to pesticides has been assessed and all these procedures are widely described in chapters III and V.

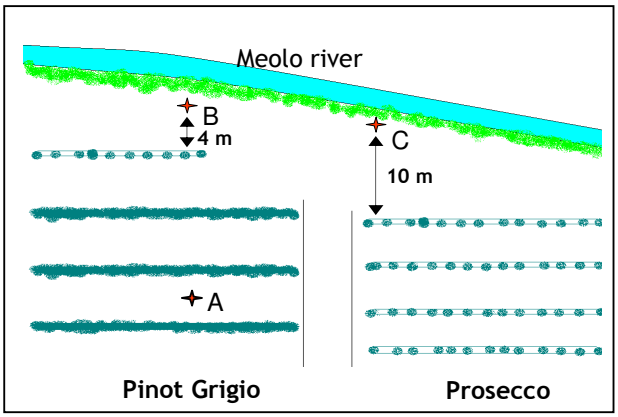

Figure 7.1. Community sampling points in the vineyard (modified after Vaj *et al.*, 2010).

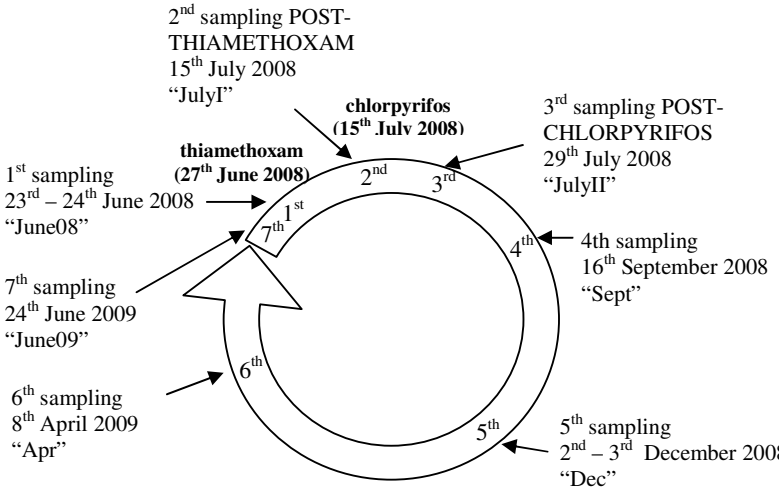

Figure 7.2. Scheme of the sampling dates (modified after Vaj *et al.*, 2010), also indicating insecticide application dates and codes of the samplings between quotation marks.

Table 7.1 shows the PPPs applied in the vineyard, their application dates, the amount used and their Henry's law constant (property thought to be related

with their relative availability in soil pore air or water). Henry's law constants were collected among the available official information.

Table 7.1. Active ingredients, application dates, total amounts applied in the vineyard and Henry's law constant (-: data not available). For repeated applications the first and the last dates in the productive season are given. Italics: a.i. applied only on Pinot Grigio variety.

a.i.	Application dates		Total amount (kg)	Henry's law constant at 25°C (Pa m^3 mol^{-1})(1)
	start	end		
sulfur	28-Apr-08 15-Apr-09	11-Aug-08 22-Jun-09	364.00 172.00	6.76E-01(1)
mancozeb	28-Apr-08	20-Jun-08	82.50	5.39E-02(2)
dimethomorph	19-May-08	27-Jun-08	5.00	1.52E-05(1)
glyphosate	20-May-08 20-Apr-09	5-Aug-08 14-Jun-09	11.65 7.20	2.10E-07(1)
oxadiazon	20-May-08		3.70	3.50E-02(1)
folpet	24-May-08 15-Apr-09	14-Jun-08 12-Jun-09	54.00 44.95	7.80E-03(1)
iprovalicarb	14-May-08	14-Jun-08	0.75	1.45E-06(1)
cyprodinil	17-Jun-08 12-Jun-09		1.05 1.05	6.90E-03(1)
fludioxonil	17-Jun-08 12-Jun-09		0.70 0.70	5.40E-05(1)
thiamethoxam	27-Jun-08 22-Jun-09		0.25 0.25	4.70E-10(1)
copper oxychloride	27-Jun-08 12-Jun-09	5-Aug-08 22-Jun-09	24.00 8.69	-
chlorpyrifos	15-Jul-08		2.25	6.76E-01(1)
copper sulphate	22-Jul-08	11-Aug-08	8.94	-
mepanipyrim	5-Aug-08		1.00	1.67E-03(1)
*thiophanate-metyl**	7-Jun-09		1.26	8.10E-05(2)
carbendazim	not applied, derives from thiophanate-methyl			3.60E-03(1)

* Partly degrades in soil into carbendazim.
(1) Tomlin, 2003; (2) FOOTPRINT, 2006.

7.2.2. Trait collection for microarthropod community

For a trait-based risk assessment, some attributes considered important for the organisms composing the sampled soil microarthropod community were identified, for assessing their vulnerability to the chemical stress present in

the vineyard. The organisms for which traits were identified were those sampled, with the same systematic detail: family level for springtails, four major groups for mites and order level for the rest of the community, with some exception (e.g. Hymenoptera: Formicidae). Thus the trait-based approach is strictly related to the field work performed in chapter V.
All traits were divided into four main categories: morphological, life cycle, physiological and ecological. Characteristics considered to be important for microarthropod community were identified and for each of them a value for each taxon was given, according to literature, expert judgment and direct observations on soil fauna. For discrete variables attributes (e.g. body shape), trait categories were identified and a value according to the affinity of the taxon with the category was given, assigning values ranging from 0 and 1. For continuous variables (e.g. body length), the trait value was range scaled using equation 7.1:

$$x'_{ij} = \frac{x_{ij} - L_j}{U_j - L_j} \qquad \text{(Eq. 7.1)}$$

where:
x'_{ij} is the new variable;
x_{ij} is the original variable;
L_j is the minimum value among the variables of the j-th row;
U_j is the maximum value among the variables of the j-th row.

All these traits and values were organised into a matrix. Using the two presented methods for assigning values to traits, the matrix was normalised between 0 and 1. The constructed matrix is presented in Appendix B. The main problem was the lack of information for soil organisms, thus the main bias of the matrix is that a lot of possible important traits were excluded because data were missing and the matrix was shaped according to the availability of the information.
The 14 characteristics will be briefly described, focusing also with their relation with vulnerability.

- Morphological traits

Traits included in this group were: body size, body shape and presence of cuticle or exoskeleton.
Body size, expressed as body length, is a continuous entry, normalized with a range scaling (eq. 7.1); the maximum body length that a taxon may assume was taken, sometimes averaged within the sub-taxa constituting the higher level. On the other hand, shape is discrete and four categories ("globular", "oval flattened", "elongated", "very elongated") were identified. Size and shape of an organism are important because they determine the

surface/volume ratio and the higher is this ratio, the higher is the probability that a substance may enter into the organism, thus the higher is the sensitivity. Also the presence of a cuticle or exoskeleton acts on this parameter (sensitivity), being a barrier to the entrance of xenobiotics into the organism via skin. The categories of this trait were: "soft body", "chitinous" (as arthropods) and "hard" (in case of calcareous cuticle, elytron or sclerified tegument).

- Life cycle traits

Life cycle characteristics were: maximum age, number of life cycles (generations) per year, life stages (instars), method of reproduction, duration of life in soil.

Maximum age that can be reached by organisms has been divided into three categories: "1-2 months", "2-12 months", "more than 12 months". Stress given by plant protection products, that can be discontinuous, affects only a part of the total life of an organism, to a minor extent the longer is the lifespan; thus a long living organism would be less vulnerable to this kind of stress. Also the number of generations per year ("one" or "more") is inversely correlated with vulnerability, acting on recovery potential, the more life cycles are, the faster is the recovery.

The number of instars was quantified using two categories: "holometabolic" (complete metamorphosis) and "not complete metamorphosis". The juveniles forms are generally more sensitive than adults, thus if an animal is holometabolic, its juvenile form, morphologically different, is considered to be more sensitive than a juvenile form more similar to adult.

Methods of reproduction were divided into two categories, "bisexual" (sexual with fertilization) and "parthenogenetic" (sexual but without fertilization), and the second one is considered to increase vulnerability, since in a stressed environment the production of clones is disadvantageous.

Being soil the compartment of the analysis, the resident period in soil has an influence on vulnerability. Generally, soil organisms can be divided into strictly edaphic (category "whole life") and those which spend in the compartment only the first life stages (category "only part"); in the first case vulnerability would be higher because the individual would spend the 100% of its life in the compartment on which the stress acts.

- Physiological traits

The only attribute for which sufficient information was available was respiration type, for which five categories were identified: "tracheae", "ventral tube", "book lungs", "pleopods" and "cutaneous". This trait is strictly related to the substance taken into account: for chemicals relatively more available in soil pore air vulnerability would be higher in presence of tracheae, for chemicals relatively more available in soil pore water organisms with cutaneous respiration would be more vulnerable.

- Ecological traits

Characteristics in this group were: food preferences, vertical stratification, vertical movements, strategies against adverse conditions (diapause or supercooling), position in trophic web.

Food preference could be important for the indirect effect of the elimination of the food sources, thus six categories were identified: bacteriophages ("bacteria"), fungivores ("fungi"), saprophages, necrophages, organisms consuming structured detritus of animal origin ("sapr/necr"), organisms consuming amorphous detritus ("detritus"), herbivores, animals feeding on leaves, roots, mycorrhizae, algae, structured vegetal detritus ("plant materials") and secondary consumers, as predators and parasites ("animals"). Also in this case the trait is related to the chemical taken into account.

The position in the trophic web, on the other side, was considered in a different trait ("1st consumers", "2nd consumers") and the vulnerability was considered to increase with the increasing of the trophic level. The values were derived from the food preferences.

Vertical stratification was considered, because in the surface layers a pesticide applied can reach directly the soil and thus the contamination could be higher than in the deeper layers. There the chemical can be transported by leaching, but during the time between application and reaching of a particular depth the substance can be degraded by several processes in soil. Anyway, physical-chemical properties of chemicals, soil characteristics which determine the mobility of a substance, as porosity, should be considered. Four categories were identified: "aboveground", "epigeic", "hemi-edaphic" and "eu-edaphic". For springtails the division was based on descriptions in literature of the organisms, that were divided into "epigeic", "hemi-edaphic" and "eu-edaphic" according to the following criteria:

- epigeic: intensely coloured, striped or dotted, well developed eyes (8+8 ocelli), long antennae and legs, big size, presence of long setae or scales, presence of long furca;
- hemi-edaphic: pale or partial coloured, partially developed eyes (from 6+6 to 1+1 ocelli), medium antennae and legs, possible presence of setae, presence of furca;
- eu-edaphic: not coloured, blind or sometimes with 1+1 ocelli, short antennae and legs, absence of furca.

Vertical movements along the soil profile constitute a possible escape from the more contaminated superficial layers. The trait categories were considered as boolean ("yes"/"no").

Although the analysis is stress-specific, in a real environment not only one stress (plant protection product applications), but a variety of them is present. Physical parameters fluctuations, as temperature or soil moisture, can create adverse conditions for soil fauna, causing multiple and cumulative stress, making the community more vulnerable also to the chemical stressor. For this reason the presence of strategies against adverse conditions, like

diapause or supercooling, is considered to decrease the vulnerability of an organism. The trait is dichotomous: "presence" or "absence".

7.2.3. Selection of significant traits

For a better identification of the microarthropod behaviour in relation to their characteristics, traits were analysed one by one, for selecting significant ones and eliminating the background noise level. For each trait a Principal Component Analysis (PCA) was performed using as dataset the mean abundance of sampled organisms for each point and each date with the values given for the trait category for each taxon.
The traits for which a particular disposition of the community in the PCA output was observed were considered as significant in determining a particular behaviour of the community in relation to the stress present in the vineyard. This consideration highlights once more that the analysis performed is site-specific.
An example is shown in figure 7.3, for the trait "vertical stratification".

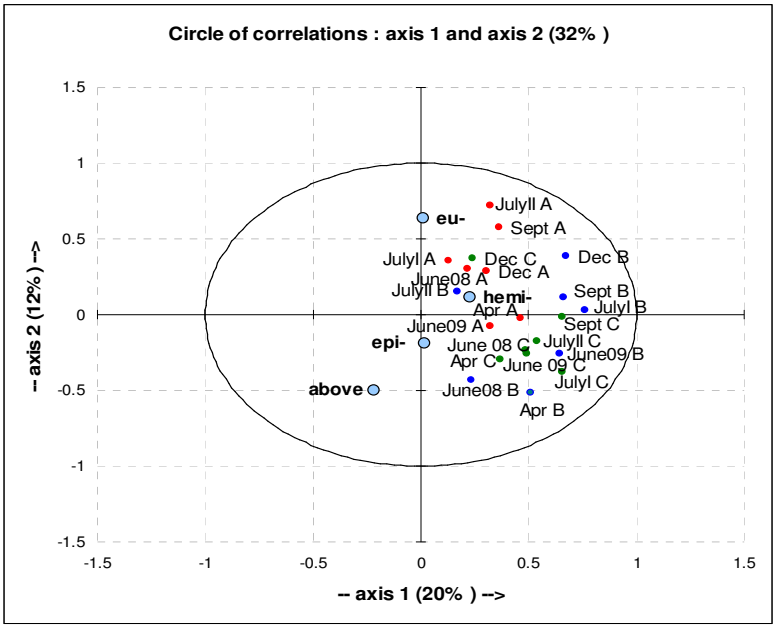

Figure 7.3. Output of the PCA performed for the trait "vertical stratification". Red dots refers to sampling point A, blue for sampling point B and green for sampling point C (for details see section 7.2.1). Bigger blue dots refers to trait categories, above: aboveground, epi-: epigeic, hemi-: hemi-edaphic and eu-: eu-edaphic.

The four trait categories lay along axis 2 from deeper layers (positive values) to surface (negative values). Also sample arrangement in the PCA follows axis 2. More contaminated samples, collected within the vineyard in insecticide post-application dates, or collected during the averse temperature conditions in winter have positive values, as "eu-edaphic" category. Samples collected inside the vineyard in pre-application or recovery dates and those collected outside the field after insecticide application have values close to 0, as the category "hemi-edaphic". Finally, less contaminated samples, outside the vineyard in pre-application or recovery dates, have negative values, as "epigeic" and "aboveground" categories. Thus in the first set of samples eu-edaphic organisms are relatively more abundant, in the second set hemi-edaphic and in the last set epigeic and aboveground.
The other seven significant traits were: maximum body length, numbers of generation per years, instars, duration of life in soil, vertical movements, strategies against adverse conditions and position in the trophic web.

7.2.4. Focus on springtail and mite traits

For springtails and mites all the 14 traits were used because the influence of some specific traits was not detected. In contrast with the analysis of the whole community, some traits were excluded because the values given to the category were not varying among taxa. An example, both for springtails and mites, is the trait "instars", for which the category "no metamorphosis" takes a value as high as 1 for all the springtail families or the mite groups, since these taxa are not holometabolous.
The traits used for springtail community were thus 8: maximum body length, body shape (only "globular" and "elongated"), maximum age (only "1-2 months" and "2-12 months"), generations per year, respiration type (only "tracheae", "ventral tube" and "cutaneous"), food preference (only "fungi", "sapr/necr", "detritus", "plant materials" and "animals"), vertical stratification (only "epigeic", "hemi-edaphic" and "eu-edaphic") and position in the trophic web.
Traits used for mite community were 8 as well: maximum body length, body shape (only "globular" and "oval flattened"), exoskeleton/cuticle (only "hard" and "chitinous"), generations per year, respiration type (only "tracheae" and "cutaneous"), food preferences, vertical movements and position in the trophic web.

7.2.5. Statistical methods: Community Weighted Means

Beside the application of Principle Component Analysis, another statistical method was used, the calculation of Community Weighted Means (CWM) for each trait (eq. 7.2):

$$CWM = \sum_{i=1}^{n} P_i \cdot Trait_i \qquad \text{(Eq. 7.2)}$$

where:
P_i is the relative abundance of i-th taxon within the community;
n is the total number of taxa considered in the community;
$Trait_i$ is the value given to the trait category taken into account, for the i-th taxon.

The two methods can be considered as complementary, because PCA is multivariate and permits an holistic view of the community behaviour in relation to traits, while CWM disaggregates the information and permits a focus on each trait. The CWM method was used to confirm or not the hypotheses arisen from PCA analyses.
Regarding other statistical methods, community counts were range scaled prior to analysis, according to equation 7.1. For each sampling date and point a medium value from the replicates was used, to minimize background noise.

7.3. Result and discussion

7.3.1. Trait based analysis of the whole community

The community behaviour in relation with the 8 significant traits was analysed with PCA. As dataset the mean abundance of sampled organisms for each point and each date and the values given for each trait category for each taxon were used (figure 7.4).

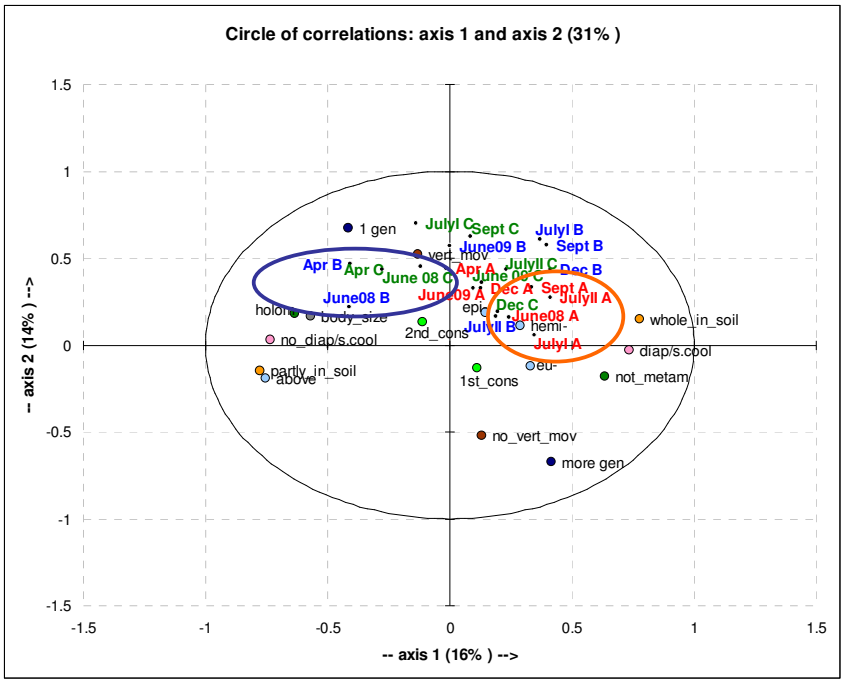

Figure 7.4. Output of the PCA performed for whole community and the 8 significant traits. Red writings refers to sampling point A, blue for sampling point B and green for sampling point C (for details see section 7.2.1). For trait category abbreviations see Appendix C. Blue circle highlights samples collected in less contaminated situation, while orange circle samples with higher pesticide presence.

Samples collected within the vineyard with higher pesticide presence (orange circle in figure 7.4) lay on the opposite side respect to those collected in pre-application dates outside the vineyard (blue circle in figure 7.4). Also some trait categories follow the same distribution in the PCA output.

The right side of the graph, related with most contaminated samples, is characterized by organisms with the following traits:

- duration of life in soil: whole life;
- presence of strategies against adverse conditions, as diapause or supercooling;
- no complete metamorphosis;
- vertical stratification: eu-edaphic and hemi-edaphic;
- more generations per year.

For the last two traits only the value on axis 1 was taken into account.

The hypothesis is that they are related to lower vulnerability.

These important traits will be briefly discussed.

Diapause/supercooling. As reported in section 7.2.2, organisms with diapause or supercooling as strategies against adverse conditions can be less vulnerable because more resistant to multiple and combined stress.
Instars. Taxa with juvenile forms similar to adults ("no metamorphosis") can be less vulnerable for a minor sensitivity of juveniles.
Vertical stratification. Regarding the vertical stratification categories ("eu-edaphic" and "hemi-edaphic"), the result is in accordance with the theoretical concept of vulnerability, because animals that live in deeper layers are far from the most contaminated part of the compartment.
Number of generations per year. Having more generations per year lead to a faster recovery, thus a minor vulnerability.
Duration of life in soil. On the other hand, some differences with the theoretical relation trait-vulnerability presented in section 7.2.2 are found. This is the case of the duration of life in soil. In section 7.2.2 it was reported that, being soil the compartment of interest, organisms living in that compartment for the whole life are more exposed to pesticide contamination in respect to those that emerge in a certain time of the year. Thus organisms living the whole life in soil should be more vulnerable. In the PCA in figure 7.4 the result is the opposite, being this trait related to those giving lower vulnerability. This result can be related to the correlation among the selected traits, that is ineradicable, except cancelling the whole trait from the analysis. Moreover, this analysis is linked to a real situation, not to sensitivity data obtained in laboratory, thus is inevitable that an attribute that makes a taxon less or more vulnerable belongs to organisms that possess also other characteristics. In this case , for example, organisms that spend the whole life in soil are also those that live in deeper layers. With these considerations it can be concluded that the duration of life in soil is an attribute that describes less vulnerable organisms, but it's not a driving force in defining vulnerability of soil microarthropods.

Similar considerations can be done for the left part of the graph. Traits that lay in that side, related to less contaminated samples are:

- holometaboly;
- absence of strategies against adverse conditions, as diapause or supercooling;
- one generation per year;
- duration of life in soil: only part;
- body length.

They should be related to higher vulnerability, because they are related to the less contaminated samples.
Diapause/supercooling, instars, number of generations per year. The first three trait categories are opposite to the attributes on the right part of the graph and it is possible to conclude that are the traits making a taxon more vulnerable. This conclusion is in accordance to the theoretical relation trait-

vulnerability. Holometabolous larvae are considered to be more sensitive compared to their counterparts without metamorphosis, taxa without diapause or supercooling can be more susceptible to multiple and combined stress and individuals with only a generation per year have a poor recovery potential.

Duration of life in soil. The duration of life in soil has been discussed previously: in this part of the graph the opposite to the right part is found for this trait and the considerations are the same.

Body length. Also body size is not in accordance with the theoretical relation with vulnerability, because smaller organisms have higher surface/volume ratio and thus higher susceptibility to exposure, and a potential higher vulnerability. In this case it seems that bigger organisms are more vulnerable. Once again the correlation among traits and the link to field situation are highlighted. Indeed, smaller organisms are also those living in deeper soil layers and less susceptible to exposure (right part of the PCA output) and bigger organisms are also those that fly and have a complete metamorphosis. Also for body size it can be concluded that it is not a driving force in determining vulnerability, although is a trait related to more vulnerable organisms.

Summarising the presented results, traits responsible to low vulnerability are the presence of strategies against adverse conditions, a not complete metamorphosis, the tendency to live in soil deeper layers and having more generations per year. On the other hand, the absence of diapause or supercooling, holometaboly and having only one generation per year are related to higher vulnerability.

Correlation among traits is also visible in the biplot of the PCA obtained using as dataset only the trait matrix, without the results of the sampling campaign, shown in figure 7.5.

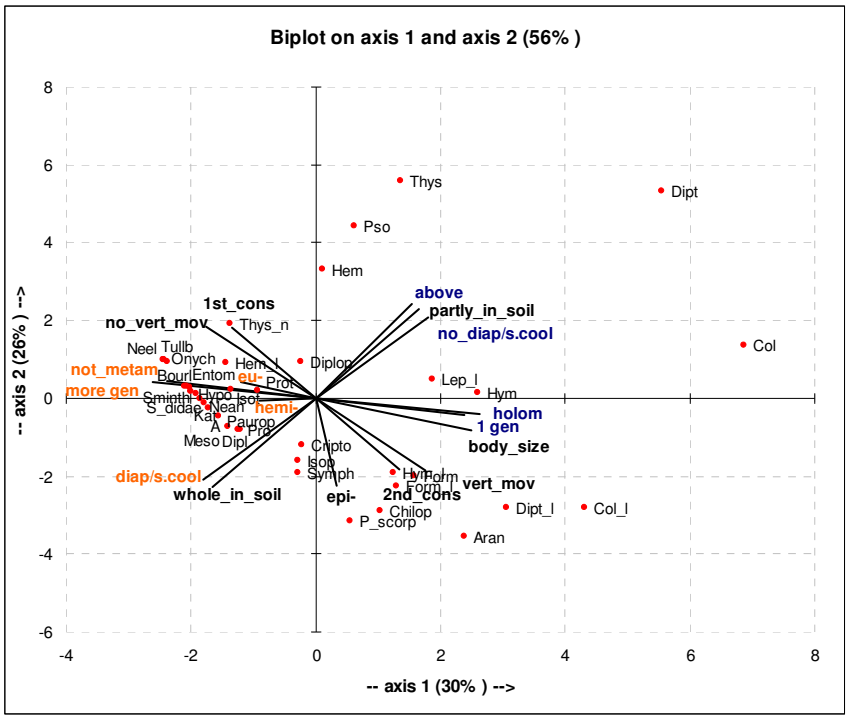

Figure 7.5. Biplot of PCA obtained from the trait matrix with only the significant traits. Traits written in orange are those that, in this site-specific analysis, are linked to lower vulnerability, while those written in blue are linked to a higher vulnerability. Red dots refer to taxa. For abbreviations see Appendix A and C.

From figure 7.5 taxa that possess attributes linked to less or more vulnerability are shown.

In the right part of the biplot, related to traits linked to higher vulnerability there are Diptera, Coleoptera, Hymenoptera, larvae of Lepidoptera and, considering mainly value on axis 1, also larvae of Diptera and of Coleoptera. Larvae of these taxa are different from adults, they do not possess strategies for adverse conditions, they fly and live mainly aboveground. Anyway, they are all taxa that in the months in which pesticides are present in soil, July and partly September, are not present in soil because of their life cycle (they emerge from soil and fly), not their vulnerability to stress. Moreover, all of these taxa, except beetles and their larvae, are considered as rare in the samples, because found sporadically.

On the other side, in the left part of the biplot, related to traits linked to minor vulnerability more edaphic taxa are found: springtails, mites (except Cryptostigmata), Pauropoda, Protura and Diplura. These taxa, in general, live in deeper soil layers, have a higher recovery potential due to having more generations per year and possess strategies against adverse conditions.

Since these characteristics are owned by all of the cited taxa, a focus was made to identify the traits possibly determining minor or major vulnerability in springtails and mites, two groups considered less vulnerable to pesticide presence in soil (sections 7.3.2 and 7.3.3).

Procedure described in section 7.2.5 were used to calculate the time trend of Community Weighted Means (CWM) for the traits driving vulnerability.
As an example the time trend of the vertical stratification is shown (figure 7.6). CWMs were calculated for the sampling points inside the vineyard and only the time trend is visible. The procedure allows to see the trend of a particular trait before and after a perturbation.
In this work CWM were used as confirmations of the hypotheses done about traits driving vulnerability. Due to the high standard deviations of the replicates, CWMs are assumed as indications.

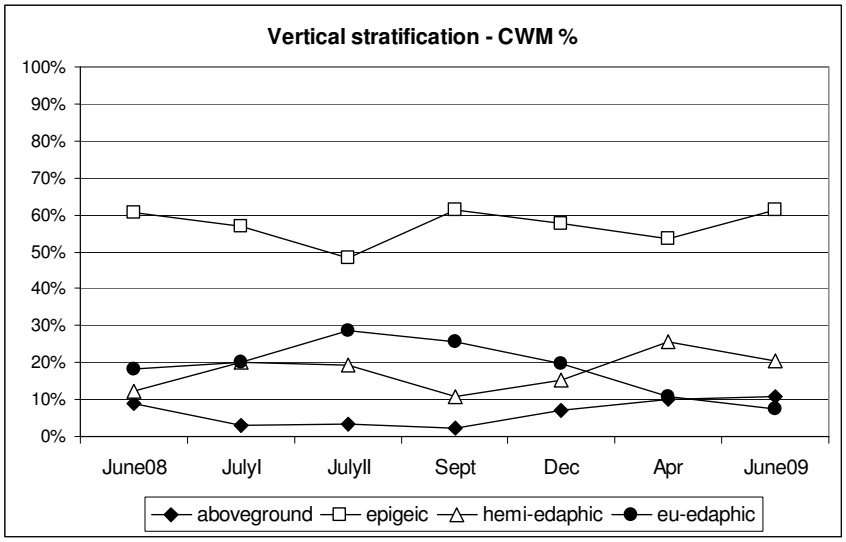

Figure 7.6. Time trend of CWM, expressed as percentages, for the trait "vertical stratification", calculated inside the vineyard (A point).

In figure 7.6 it is shown that the trait category "epigeic" is slightly decreasing after chlorpyrifos application (between JulyI and JulyII), indicating that the trait category is affected by this kind of contamination. On the other hand, eu-edaphic category increases after stress. This confirms what observed in the PCA result (figure 7.4): epigeic organisms are more affected by pesticides and eu-edaphic are less affected. It may be concluded that vertical stratification can be an important trait in determining vulnerability.
For the other traits the result and a brief discussion are reported below.

For holometaboly and diapause/supercooling the trends are in accordance with the PCA results.
The trend of organisms with only one generation per year, on the other hand, is partly in disagreement with the previous analysis. It is found to decrease between June08 and JulyI, with fungicide applications, but it increases between the two dates of July, when it is supposed to decrease, according to the fact that is related with high vulnerability. Anyway, organisms with only one generation per year are always relatively low respect their counterparts with more generations. These organisms, indeed, are always in relatively high numbers and show a decrease during summer applications of insecticides. Anyway, having more generations per year seems to be a favoured trait in the months in which recovery is more important, from JulyII and September and from April to June09 (community resume after winter). Anyway, standard deviations are high, thus these trend should be seen just as indications.

7.3.2. Trait based analysis for Collembola

Traits presented in section 7.2.4 were use for performing a PCA together with the sampling results for springtail families. The output is shown in figure 7.7. Several PCA were made, eliminating one trait each time and using different combinations, but this was the one giving the best result as resolution and minimum background noise.

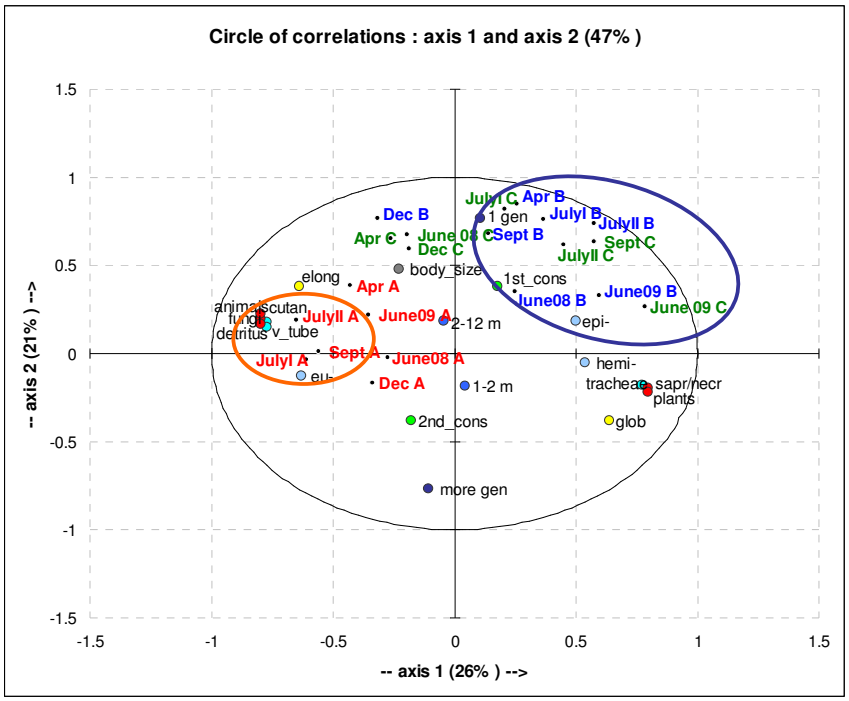

Figure7.7. Output of the PCA performed for springtail community and traits. Red writings refers to sampling point A, blue for sampling point B and green for sampling point C (for details see section 7.2.1). For trait category abbreviations see Appendix C. Blue circle highlights samples collected in less contaminated situation, while orange circle samples with higher pesticide presence.

As for the whole community (section 7.3.1), also for springtails a clear distribution of samples is evident.

On the left part of the graph the samples collected inside the vineyard in post-application dates and thus more contaminated are present. The same side of the graph is also characterised by springtails with the following traits:

- elongated body shape;
- eu-edaphic;
- respiration type: mainly ventral tube and skin;
- food preferences: animal, fungi and detritus.

The hypothesis is that these traits are related to lower vulnerability.

Vertical stratification. As for the whole community, deeper layers can be less contaminated, at different extents depend on the mobility, thus the animals are less exposed to pesticides.

Respiration type and food preference are related to the chemical properties.

Respiration type. In the vineyard different pesticides are applied, with different properties that can make them relatively more available in soil pore

air or water (as the Henry's law constant listed in table 7.1). Respiration through ventral tube or skin makes the organism less vulnerable to chemicals relatively available in air, being respiration through tracheae the main way for them to enter the body.
Food preference. In the vineyard, insecticides, herbicides and fungicides are used. Thus the food category "animals", "plants" and "fungi" should be related to less contaminated samples. However, it should be noted that no one of these families feed on only one source and maybe the food web is well structured enough to permit different sources consumption when one is depleted. Thus this trait seem more due to a correlation among traits and describes less vulnerable families, without being a driving force for them.
Body shape. A similar consideration can be done for the elongated shape, that should be related to the less impacted sites, being a characteristic that increases the vulnerability, increasing the surface/volume ratio. It should be noted that a big proportion of elongated organisms found in the samples are also those living in the deeper layers (e.g. some isotomids, or almost all the families in the group Poduromorpha), while, on the other hand globular organisms were also epigeic, as e.g. the majority of Symphypleona.

On the right part of the graph less contaminated samples are found, those collected outside the vineyard. Trait categories found in this part of the graph are:

- globular shape;
- vertical stratification: epigeic and hemi-edaphic;
- respiration type: tracheae;
- food preferences: saprophages/necrophages and plant consumers;
- position in trophic web: primary consumers;
- one generation per year.

Vertical stratification. Organisms living in the surface layers (epigeic and, although less, hemi-edaphic) are those more exposed to contamination.
Number of generation per year. Having only one generation per year leads to a minor recovery potential.
Respiration type. Respiration through tracheae makes an organism more exposed to chemicals relatively more available in soil pore air.
So, these traits should indicate higher vulnerability.
Food preference. Traits related to food preferences have been discussed previously and they may describe organisms, without being responsible of the vulnerability.
Position in trophic web. The position in the trophic web seems to be related to the food preference towards animals, that is linked to less vulnerable families.
Body shape. For globular shape the same considerations made for elongated springtails can be done, thus this trait can be considered just an attribute of

more vulnerable families, but not a driving force for the higher vulnerability (e.i. they are more vulnerable because are epigeic and the majority of epigeic springtails found was globular).

Summarising the presented results, traits giving a minor vulnerability may be the tendency to live in deeper layers and, for chemicals relatively more available in soil pore air, the respiration type more related to ventral tube and skin, while traits responsible to a higher vulnerability may be the tendency of living in the superficial soil layers, respiration through tracheae and having just one generation per year (only some organisms in the family Isotomidae).

Correlations among traits is visible in the biplot obtained using as dataset only traits for Collembola (figure 7.8).

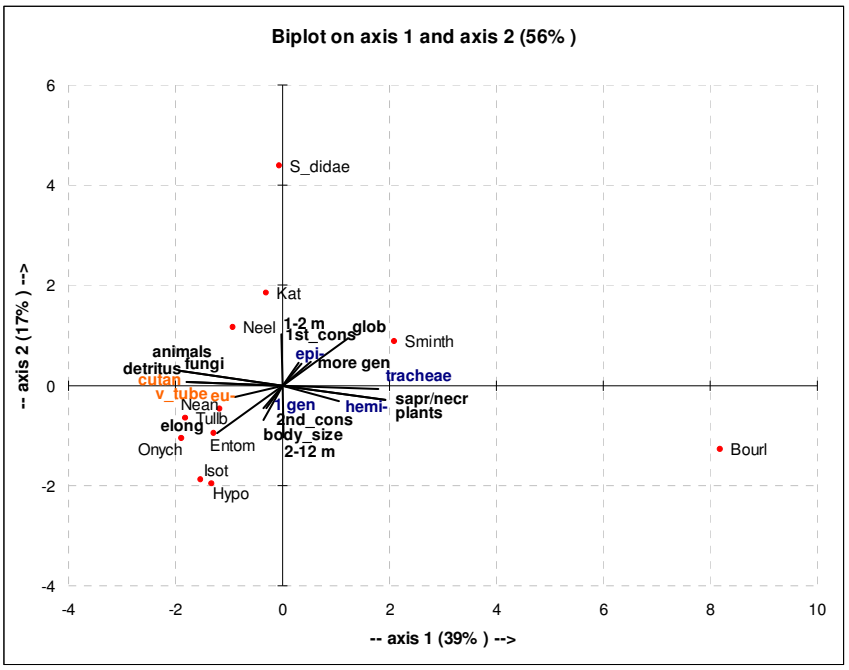

Figure 7.8. Biplot of PCA obtained from the trait matrix with only traits for springtails. Traits written in orange are those that, in this site-specific analysis, are linked to lower vulnerability, while those written in blue are linked to a higher vulnerability. Red dots refer to springtail families. For abbreviations see Appendix A and C.

Families linked to traits that make them less vulnerable, and thus considered less vulnerable themselves in this site-specific analysis, are mainly Neannuridae and Tullbergiidae, both within the suborder Poduromorpha,

small, elongated and mainly eu-edaphic. Also Entomobryidae and Onychiuridae are in the same part of the graph, at least for axis 1 values.
Linked to traits giving higher vulnerability and thus considered more vulnerable, are Sminthuridae and Bourletiellidae, two epigeic globular families within the suborder Symphypleona. In the trait category "one generation per year", that is considered to lead to higher vulnerability, only Isotomidae have a value above 0, but this family has other traits that makes it not so vulnerable.

Time trend of CWMs calculated for traits that drives springtail vulnerability confirm clearly the results for the vertical stratification. For the other two traits the numbers are too low to detect a clear indication of variability considering only time or only space.

7.3.3. Trait based analysis for mites

A PCA for mites was performed using the results of the counts in the samples and the traits presented in section 7.2.4. The output is shown in figure 7.9.

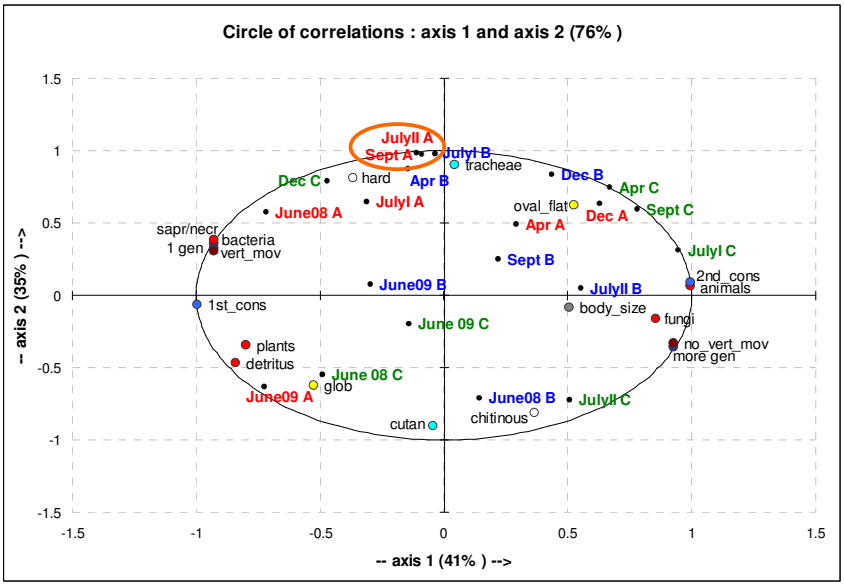

Figure 7.9. Output of the PCA performed for mite community and traits. Red writings refers to sampling point A, blue for sampling point B and green for sampling point C (for details see section 7.2.1). For trait category abbreviations see Appendix C. Orange circle highlights samples collected with higher pesticide presence.

In figure 7.9 only a small cluster with the sampling points with higher pesticide presence is detected. Traits linked to this cluster are the presence of a hard cuticle and a respiration type using tracheae.
These traits should be responsible of a minor vulnerability to the stress.
Cuticle. The presence of a cuticle is theoretically linked to a minor vulnerability, because it acts as a shield for the contact of an organism with a chemical.
Respiration type. Respiration through tracheae should make an organism more vulnerable to chemical relatively available in soil pore air and less vulnerable to those likely to be present in soil pore water.

Figure 7.10 shows the biplot obtained performing a PCA only with traits of mites.

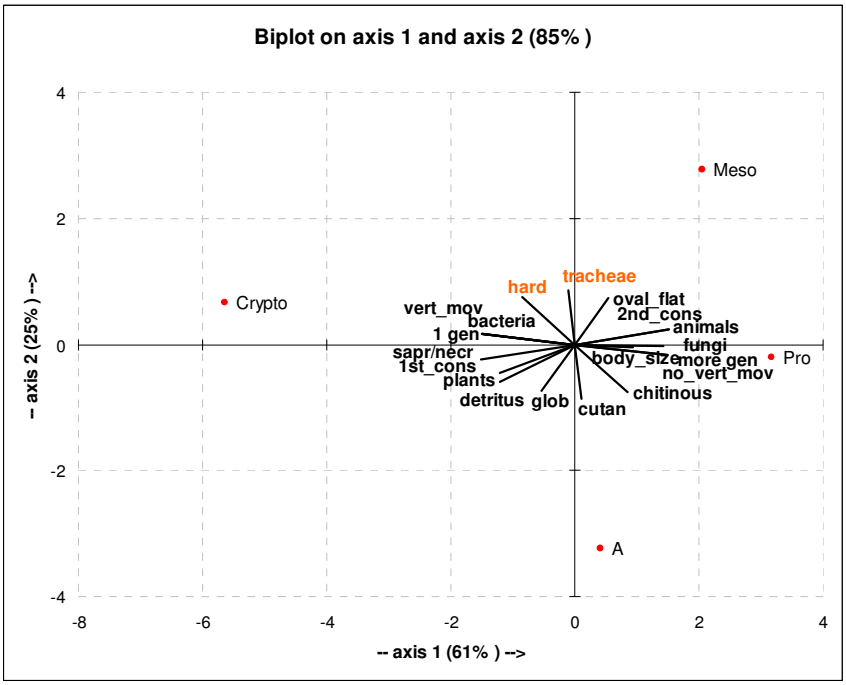

Figure 7.10. Biplot of PCA obtained from the trait matrix with only traits for mites. The only trait written in orange is the one responsible for a minor vulnerability. Red dots refer to mite groups. For abbreviations see Appendix A and C.

The groups with a hard cuticle, that is responsible for a minor vulnerability are Cryptostigmata and, in part, Mesostigmata. Also respiration through tracheae, giving a low vulnerability to chemicals relatively available in soil pore water, gives the same result. The result differs from what observed in the whole community trait based analyses, where Cryptostigmata were not

considered in the less vulnerable group. It should be noted that in the analysis focused on mites a lot of background noise for this group has been eliminated by eliminating other taxa. Moreover, and this seems more important, the trait matrix for mites was shaped on the information with variation, thus excluding some traits, and including traits that were not significant for the whole community. Including all the traits in the whole community analysis would have led to a too high background noise and too low resolution in the analysis, while for the mite community this was not a problem because of the low number of taxa entries. Anyway, we consider more valid the analysis focused on the group (mites or springtails) on which differences among sub-taxa can be observed because of a better resolution.

Time trend of CWMs calculated for the presence of a cuticle or exoskeleton confirms this result.

7.3.4. Comparison with traditional trend analyses

In this section a comparison between the three trait based analyses performed, on the whole community, on springtails and on mites, and the traditional trend analyses shown in chapter V (section 5.3.1) is reported. In chapter V it was possible to observe that some taxa were more affected by pesticide presence (Isotomidae, Katiannidae and astigmatid mites) and others were favoured by the pesticide applications (Hypogastruridae, Tullbergiidae, Neelidae, Pauropoda and Diplopoda). Higher or lower vulnerability was taken into account to explain this behaviour.
Trait based analysis confirms these conclusions for pauropods and Tullbergiidae, considered as less vulnerable to pesticide presence in both the analyses.
However, some differences in the results arise.
For mites, traditional analysis highlighted a possible vulnerability of Astigmata that is not clear in the trait based one. Anyway, it should be noted that astigmatid mites in the biplot shown in figure 7.10 lay in the opposite part of the traits giving a minor vulnerability. This is not clear in the output of the PCA, because a cluster with attributes linked to high vulnerability was not detected, but can be an indication of concordance between the two results. On the other hand Cryptostigmata have been shown to be less vulnerable in the trait based analysis, but no indications on this behaviour arose in chapter V.
Regarding springtails, from traditional trend observation it seems that Katiannidae and Isotomidae are more vulnerable, but the result is not confirmed here. Anyway, the first family is strongly epigean, and the second is the only one having part of the organisms with one generation per year (figure 7.8) and these traits are responsible of a higher vulnerability.

A possible cause of the difference can be the incompleteness of the trait based analysis. Firstly, even if the trait identification is based on sound principles and a literature study, it contains an element of judgement, thus some important attributes could be excluded. Improvements in defining possible characteristics to be included in the analysis could lead to a better completeness. Then, and maybe more important, trait matrix was shaped on available information, thus many traits were excluded from the analysis because of the lack of some data. This seems to be one of the major obstacles in a trait based risk assessment for soil compartment.

7.4. Conclusions

From the trait based analyses it was possible to detect traits considered as driving forces in determining the vulnerability of organisms. Three different analyses were done, on community and focusing on springtails and on mites.
For the whole community traits considered as driving forces were:

- vertical stratification,
- instars,
- strategies against adverse conditions,
- number of generations per year.

This last trait, anyway, is partly controversial, due to a second analysis of the time trend of the CWM.
Other traits were linked to higher or lower vulnerability: duration of life in soil and size. Anyway, they are just considered to describe organisms, but not to be driving forces of their vulnerability.
More vulnerable taxa, holometabolic and without strategies as diapause or supercooling, were Diptera and Coleoptera and their larvae, Hymenoptera and Lepidoptera larvae. Anyway these taxa, except beetles and their larvae, are considered as rare in this field work, thus a conclusion about their vulnerability cannot be made.
Less vulnerable taxa, those living in deeper layers, with diapause or supercooling and without a complete metamorphosis, were springtails, mites (excluding Cryptostigmata), Pauropoda, Protura and Diplura.
Focusing on springtails, three traits were considered as driving forces for vulnerability:

- respiration type (depending on relative availability of chemicals in soil pore air),
- vertical stratification,
- number of generations per year.

Diet and body shape were identified as attributes just describing families with high or low vulnerability.

Vulnerable families are considered to be Sminthuridae and Bourletiellidae, while on the other side Neanuridae, Tullbergiidae and partly Entomobryidae and Isotomidae are found.
The results of the field work presented in chapter V can be explained by these results.
The traits determining vulnerability of mites are:

- presence of a hard cuticle,
- respiration type (depending on relative availability of chemicals in soil pore water),

Thus less vulnerable organisms were Cryptostigmata and partly Mesostigmata. Anyway, the results of the field work in chapter V can be described using the results of the trait analysis.
Differences among the three different trait based analyses in defying vulnerable taxa were explained by differences in trait matrices used and the high background noise level for springtails and mites when the whole community is considered. Anyway, for each level of resolution only a few traits were responsible of the vulnerability and they were in accordance.
It should be noted that the analysis is related to a field campaign, thus results are strictly site-specific.
A big obstacle in this work has been identified in the lacking of information available for soil organisms, because for some traits data were not available and the whole trait had to be excluded from the analysis. Shaping the matrix on availability of data could have led to a bias.
Anyway, the trait based approach seems to be promising, being a sound tool for explaining different trends in a community behaviour.

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References

Auclerc A, Ponge JF, Barot S, Dubs F, 2009. Experimental assessment of habitat preference and dispersal ability of soil springtails. *Soil Biol Biochem* 41: 1596-1604.
Baird DJ and Van den Brink PJ, 2007. Using biological traits to predict species sensitivity to toxic substances. *Ecotox Environ Saf* 67: 296-301.

Baird DJ, Rubach MN, Van den Brink PJ, 2008. Trait-based ecological risk assessment (TERA): the new frontier? *Integrated Environmental Assessment and Management* 4: 2-3.

De Lange HJ, Lahr J, Van der Pol JJC, Wessels Y, Faber JH, 2009. Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. *Environ Toxicol Chem* 28: 2233-2240.

FOOTPRINT website, 2006. The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.eu-footprint.org/ppdb.html (accessed in December 2010).

Jørgensen HB, Hedlund K, Axelsen JA, 2008. Life-history traits of soil collembolans in relation to food quality. *Appl Soil Ecol* 38: 146-151.

Liess M and von der Ohe PC, 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environ Toxicol Chem* 24: 954-965.

Liess M, Schäfer RB, Schriever CA, 2008. The footprint of pesticide stress in communities – Species traits reveal community effects of toxicants. *Sci Total Environ* 406: 484-490.

Makkonen M, Berg MP, van Hal JR, Callaghan TV, Press M, Aerts R, 2011. Traits explain the responses of a sub-arctic Collembola community to climate manipulation. *Soil Biol Biochem* 43: 377-384.

Siepel H, 1995. Application of microarthropod life-history tactics in nature management and ecotoxicology. *Biol Fertil Soils* 19:75-83.

Tomlin, CDS (Ed). *The pesticide manual: a world compendium (13th ed.)*. British Crop Protection Council, Farnham, UK, 1606 pp.

Tranvik L, Bengtsson G, Rundgren S, 1993. Relative abundance and resistance traits of two Collembola species under metal stress. *J Appl Ecol* 30: 43-52.

Vaj C, Van Gestel CAM, Vighi M, 2010. Year-round monitoring of soil microarthropod communities under pesticide application and other stressors in a real case. Presented to SETAC Europe XX meeting "Science and technology for environmental protection", 23rd – 27th May 2010, Seville, Spain.

Vighi M, Finizio A, Villa S, 2006. The Evolution of the Environmental Quality Concept: From the US EPA Red Book to the European Water Framework Directive. *Environ Sci Pollut R* 13: 9-14.

CHAPTER VIII

Vulnerability analysis for microarthropod community under chemical stress by pesticides

Abstract

Ecological vulnerability has been introduced in ecotoxicology as a new tool to overcome the lacking in ecological realism of the traditional methods used for assessing ecotoxicological risk. It has been defined as function of sensitivity, susceptibility to exposure and recovery potential. These components can be assessed from biological traits of organisms.
A vulnerability analysis was performed on a real case, a microarthropod community under stress by pesticides. All the organisms of the community were identified at different taxon level and information on biological traits was collected. The relationship trait-vulnerability was defined and quantified through scores. For each taxon its vulnerability to single chemical and mixture was assessed through the combination of scores and trait values, using an index modified from Ippolito *et al.* (2010). Differences among the results are discussed and a comparison with the trait-based analysis is performed.

Keywords: vulnerability, traits, microarthropods, pesticides.

8.1 Introduction

In natural and anthropized ecosystems, populations of organisms may be affected by several kind of stressors. Ecotoxicological risk assessment procedures aim to identify effects on ecosystems given by the presence of potentially dangerous chemicals in a compartment. It has been highlighted in the previous chapters and in the recent literature that this approach has a great value for regulation and management, but lacks in ecological realism (Calow, 1998; Chapman, 2002, Van Straalen, 2003; Vighi *et al.*, 2006; Van den Brink, 2008). A recent tool used to overcome this lack, the trait-based analysis, was described and used in chapter VII. The method uses species attributes to predict their vulnerability to toxicants. Ecological vulnerability of species is thus a topic that is increasing in importance within the modern ecotoxicology, in particular for site-specific risk assessment. Vulnerability can be determined by three factors: sensitivity, susceptibility of exposure and recovery potential (Van Straalen, 1994; De Lange *et al.*, 2009). Sensitivity is determined by the intrinsic properties of an organism and can be estimated with an ecotoxicological endpoint, as EC_{50} (concentration determining an effect on 50% of the population). Anyway, to have a direct effect, a chemical should enter the body of an organism. In this sense susceptibility to exposure is defined, as the likelihood of an organism to be in contact with a substance or a stressor. Finally, recovery potential is the capacity of a population to recover after the disturbance, when the stress ceases.
All the three components of the ecological vulnerability can be assessed by using biological traits of the species (De Lange *et al.*, 2009). This approach thus takes into account all the advantages of using traits instead of experimental sensitivity data of species. For example, information on biological traits is also available for those species not used as indicators in risk assessment procedures and for which, thus, ecotoxicological data are not commonly available.
Ecological vulnerability is a stress specific concept, because it is strictly related to the kind of stress, sometimes even the chemical, present in the compartment. Indeed a species characteristic can be affected by a kind of stressor, but be neutral or even an advantage for others. The first ecologically relevant level that reacts to the perturbation is the population. The upscaling to community level is a function of population responses and interspecific interactions. This is a critic point for a community vulnerability analysis (Ippolito *et al*., 2010).
Though ecological vulnerability is a promising concept and is used in modern ecotoxicology, a few studies are available in literature, especially on the soil compartment. A review is given by De Lange *et al.* (2010).

In this chapter an ecological vulnerability analysis was performed for a microarthropod community present in field. The work was not general, but

related to the field work performed within the master case of the NO MIRACLE (Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe) project of the European Commission presented in chapter V. For this work not the abundances of the organisms found in field were taken into account, but their presence or absence. Thus the taxa present in the community were the same as the field work, with the same resolution: order level, except ants, springtails (family level) and mites (divided into four major groups). The traits identified and described in chapter VII and their quantification were used to describe these organisms. Being the upscaling to the community level a critic point that needs more information, the analysis was performed on populations.

8.2. Materials and methods

8.2.1. Relationships between traits and vulnerability

The set of traits identified in chapter VII (section 7.2.2) was used in the analysis. Trait groups were changed from the four used in the trait based analysis (morphology, life cycle, physiology and ecology) into the three components of vulnerability: sensitivity, susceptibility to exposure and recovery potential. The new groups are shown in table 8.1.

Table 8.1. Traits presented in the trait based analysis (chapter VII) grouped according to the components of vulnerability.

SENSITIVITY	SUSCEPTIBILITY TO EXPOSURE	RECOVERY POTENTIAL
body length	maximum age	generations per year
body shape	duration of life in soil	reproduction methods
exoskeleton/cuticle	food preferences	
respiration type	vertical stratification	
diapause/supercooling	vertical movements	
instars	position in trophic web	

In the first component, sensitivity, all the morphological and physiological traits were grouped, including the presence of strategies against adverse conditions and instars. Body size and shape determine the surface/volume ratio of an organism, thus the probability that a substance may enter the body via skin and thus the sensitivity. Also the presence of an exoskeleton or a cuticle acts on the same parameter, being a possible barrier to xenobiotics. Also respiration type is related to the probability of a chemical to enter into the organism. In a real environment, a variety of stressors can be present. Possible diapause or supercooling make an organism less sensitive to a cumulative stress given by physical stressors in addition to the chemical

ones. Regarding instars, juvenile forms are generally more sensitive than adults, thus a juvenile different to the adult form (holometabolous) could be considered to be more sensitive than its counterpart without a complete metamorphosis.
For the susceptibility to exposure all the traits related to behaviour and movements were taken into account. Age and duration of life in soil (the compartment of interest) act on the probability of an organism to be exposed to a chemical. Vertical stratification is related to the possible different contamination of different soil layers, while vertical movements could be an escape route from the contamination. Position in the trophic web drive the exposure from food source. Diet is mainly related to an indirect effect (depletion of food source) dependent on the exposure.
For recovery potential the attributes linked to reproduction were included (number of generations per year and reproduction methods).

For each trait the theoretical relationship with vulnerability, described in chapter VII (section 7.2.2) was identified and is briefly reported. When the relation is indicated as "direct" it means that vulnerability increases, as "indirect" vulnerability decreases.

- Body length: indirect relation with vulnerability, being smaller organisms more sensitive, for their surface/volume ratio;
- Body shape: categories ordered in a direct relation ("globular", "oval flattened", "elongated", "very elongated"), because the trait acts on surface/volume ratio;
- Exoskeleton/cuticle: categories ordered in a direct relation ("hard", "chitinous", "soft") because it can act like a shield to the entrance of toxicants into the body;
- Respiration type: its role on vulnerability depends on chemical properties. For substances relatively more available in soil pore air the category order for a direct relation is: "tracheae" or "book lungs", "ventral tube", "pleopods" or "cutaneous". For chemicals relatively more available in soil pore water the order is reversed;
- Diapause/supercooling: vulnerability increases from "yes" to "no", because it can prevent multiple and cumulative stress;
- Instars: indirect relation with vulnerability from "holometabolic" to "no metamorphosis", because a juvenile similar to adult form is considered to be less sensitive;
- Maximum age: indirect relation with vulnerability, because with a discontinuous stress the more the organism live, the minor percentage of its life is affected by the stress;
- Duration of life in soil: direct relation from "partly in soil" to "whole in soil", because soil is the investigated compartment and emergence is considered as an escape route;

- Food preferences: its role on vulnerability depends on chemical types. Six food categories were identified: bacteria, fungi, dead bodies or excrements or structured detritus of animal origin, plant materials, animals (for predators and parasites). For insecticides vulnerability is higher for category "animals", for fungicides for "fungi" and for herbicides for "plants". The category "detritus" is taken into account for a possible exposure via ingestion;
- Vertical stratification: indirect relation in the order "aboveground", "epigeic", "hemi-edaphic" and "eu-edaphic", because contamination after application is higher in the superficial soil layers;
- Vertical movements: vulnerability increases from "yes" to "no", because they are considered as an escape route towards less contaminated soil layers;
- Position in trophic web: direct relation from "1st consumers" to "2nd consumers", because of possible biomagnification processes;
- Generation per year: indirect relation from "one" to "more", because the second category has a faster recovery (r strategy);
- Reproduction methods: vulnerability increases from "bisexual" to "parthenogenetic", because in a stressed environment the production of clones is disadvantageous.

8.2.2. Quantification of vulnerability

For the quantification of vulnerability, an equation modified from Ippolito *et al.* (2010) was used (eq. 8.1). It was developed for ecosystem and here is applied to taxon level:

$$V_i = \frac{Se_i \cdot Su_i}{1 + R_i} \qquad \text{(Eq. 8.1)}$$

where:
V_i is vulnerability of the i-th taxon to stress;
Se_i is a value given to the influence of stress on i-th taxon sensitivity;
Su_i is a value given to the influence of stress on i-th taxon susceptibility to exposure;
R_i is a value given to the influence of stress on i-th taxon recovery potential.

For all taxa, a score ranging from 0 to 3 (0: no influence, 1: low influence, 2: medium influence, 3: high influence) was given to each category of each trait, according on its influence on the vulnerability component to which it has been linked (section 8.2.1). A score ranging from 0 to 3 is the only applicable, because the current state of knowledge in this topic allows only

an identification of high/medium/low/no influence. For traits in recovery potential group the attribution of scores is reversed (3: no influence, 2: low influence, 1: medium influence, 0: high influence), because lower values of the variable *R* lead to higher result for *V*.
In the trait matrix (Appendix B), for each taxon, a trait category has a value ranging from 0 to 1, due to the methods for attributing values used (range scaling for continuous variables and the affinity to the category for other variables). Each trait category value was multiplied by the score assigned to it. Within each component, for every taxon, results were summed, giving the three variables *Se*, *Su* and *R* in equation 8.1.
For sensitivity, a possibility for quantification would be the use of ecotoxicological tests results. For soil microarthropods, as highlighted in chapter III, they are available only for a few species, incomplete or not homogeneous. For this reason, a trait based approach in quantifying vulnerability was applied also for this component.

For quantifying the influence of ingestion and respiration in soil pore air or water, some properties of the chemicals (collected from the official available information) were considered for a set of active ingredients used in a vineyard (see chapter III) (table 8.2).

Table 8.2. Active ingredients (a.i.) present in the and Henry's law constant (-: data not available).

a.i.	Log K_{OW}	Henry constant at 25°C H (Pa m^3 mol^{-1})	K_{AW} (dimensionless) at 25°C calculated from H
chlorpyrifos	5.261[(1)]	6.76E-01[(2)]	2.78E-04
copper oxychloride	-	-	-
copper sulphate	-	-	-
cyprodinil	4[(1)]	6.90E-03[(2)]	2.83E-06
dimethomorph	2.63[(1)]	1.52E-05[(2)]	6.24E-09
fludioxonil	4.12[(1)]	5.40E-05[(2)]	2.22E-08
folpet	3.63[(1)]	7.80E-03[(2)]	3.20E-06
glyphosate	-3.2[(3)]	2.10E-07[(2)]	8.62E-11
iprovalicarb	3.2[(2)]	1.45E-06[(2)]	5.95E-10
mancozeb	0.26[(2)]	5.39E-02[(3)]	2.21E-05
oxadiazon	4.91[(2)]	3.50E-02[(2)]	1.44E-05
sulfur	-	5.00E-02[(3)]	2.05E-05
thiamethoxam	-0.13[(2)]	4.70E-10[(2)]	1.93E-13

(1) Verro *et al.*, 2009; (2) Tomlin, 2003; (3) FOOTPRINT, 2006.

Firstly, some considerations on the partitioning or binding to organic carbon soil inorganic particles were made. As a rough estimation of a trigger value, the indications proposed by the Technical Guidance Document on risk assessment (TGD; EC, 2003) about the use of the Equilibrium Partitioning

(EP) concept on sediments were followed. TGD indicates an increasing factor in using EP for substances with a Log K_{OW}>5 or with a corresponding adsorption or binding behaviour. Thus glyphosate, as a cation, was considered to bind to soil inorganic particles. For inorganic chemicals Log K_{OW} is not available, thus their water solubility was taken into account. Copper oxychloride and sulphur, due to their low water solubility values (Tomlin, 2003), were considered to adsorb on soil, while copper sulphate, being very soluble (Tomlin, 2003), was considered to have an high affinity for the water compartment. Being ingestion a possible route of exposure for chemicals bound to soil, the score given to the food preference category "detritus" was set to the maximum value. If the chemical is bind to soil, it can be considered as less available in pore water or air. Thus, for these substances, scores of all the category of "respiration type" were reduced by 1 unit.
For assessing scores in "respiration type", Henry's law constant (H) was taken into account. Dimensionless Henry's law constant (K_{AW}) was calculated from H using the following equation 8.2 (Mackay, 2001):

$$K_{AW} = \frac{H}{RT} \qquad \text{(Eq. 8.2)}$$

where:
R is the universal gas constant (8.314 Pa m^3 mol^{-1} K^{-1});
T is the temperature (K), set to 25°C = 298 K.

According to Jury *et al.* (1984) and Clendening *et al.* (1990) a pesticide could be considered as "volatile" if its K_{AW} is much higher than 2.5×10^{-5}, and "non volatile" if its K_{AW} is much smaller than 2.5×10^{-5}. Thus we considered as more available in soil pore air chemicals with $K_{AW} > 1 \times 10^{-4}$, as more available in soil pore water chemicals with $K_{AW} < 1 \times 10^{-5}$, and as intermediate chemicals with $1 \times 10^{-4} \leq K_{AW} \leq 1 \times 10^{-5}$. For copper oxychloride and copper sulphate H is not available. They were considered as more available in water, due to their negligible volatility (Tomlin, 2003).
For substances relatively more available in air a score as low as 1 was assigned to respiration types related to water (cutaneous, pleopods, ventral tube) and a score as high as 3 to respiration types related to air (tracheae and book lungs). For chemicals relatively more available in water a score as high as 3 was given to respiration types related to water and a score as low as 1 to respiration type related to air. For intermediate substances a score as high as 2 was given to all the categories. For chemicals bound to soil particles, scores were reduced by 1 unit, as previously described.

Using this procedure, because a score equal to 0 was assigned only for two traits (respiration type and food preference), *Se* ranges from 5 to 18, *Su* from

5 to 18 and *R* from 6 to 2. *V* ranges from 3.57 (taxon not vulnerable to stressor) to 108 (taxon highly vulnerable to stressor). The result is not an absolute value of vulnerability, but relative number that permits a ranking of taxa according to their vulnerability.

8.2.3. Vulnerability to a mixture of plant protection products

In agroecosystems not only individual plant protection products (PPP) are applied, but, as highlighted in chapter III, a mixture of chemical is present. As an example of real situation, exposure assessed in chapter III was used, in the same date of the risk assessment performed. The chemicals present in soil in that date (14 days after chlorpyrifos applications) are listed in table 8.3.
Time Weighted Averages (TWA) 14 days before the selected date have been calculated in chapter III, taking into account repeated applications and possible additional inputs by rainfall, for the substances shown in table 8.2. The calculated TWAs for all the chemicals were summed together, and the proportion of each one in the sum was calculated and expressed as percentage (table 8.3).

Table 8.3. Active ingredients (a.i.) present in a vineyard in late July. The situation refers to the field work presented in chapter III and here just taken as example. F: fungicide, H: herbicide, I: insecticide.

Action	Active Ingredient	Proportion(%) in the sum of TWAs
F	mancozeb	0.01%
F	sulfur	73.8%
F	dimethomorph	2.5%
H	glyphosate	4.4%
H	oxadiazon	2.8%
F	folpet	0.9%
F	iprovalicarb	0.2%
F	cyprodinil	0.5%
F	fludioxonil	0.2%
I	thiamethoxam	0.2%
F	copper oxychloride	11.5%
I	chlorpyrifos	1.1%
F	copper sulphate	2.0%

For each taxon, its vulnerability value for an active ingredient (a.i.) was multiplied by the proportion of the active ingredient in the mixture. The results were summed for obtaining a vulnerability value referred to mixture for each taxon (eq. 8.3):

$$V_{mix,i} = \sum_{j=1}^{n} V_{j,i} \cdot p_j \qquad \text{(Eq. 8.3)}$$

where:
$V_{mix,i}$ is the vulnerability of the i-th taxon to the mixture;
$V_{j,i}$ is the vulnerability of the i-th taxon to the j-th chemical;
n is the total number of chemical in the mixture;
p_j is the proportion of the j-th chemical in the compartment, compared to the others n chemicals.
The meaning of this procedure is not giving an absolute value of concentration present in the compartment. It is just a procedure to weight vulnerability according to pesticide presence in the environment.

8.3. Results and discussion

8.3.1. Vulnerability to single chemicals

Firstly, stress given by a single chemical was considered. The complete procedure is shown, as an example, for chlorpyrifos.
For each taxon considered, scores were given according to section 8.2.1. For the attribute "respiration type" Log K_{OW} and calculated K_{AW} were taken into account (see table 8.2). The chemical was thus considered with affinity for soil and relatively more available in the air fraction. Maximum score value was given for the food preference category "detritus"; a score as high as 2 was assigned to the respiration types "tracheae" or "books lungs", and 0 to "ventral tube", "pleopods" and "cutaneous". For food preference a score as high as 3 was also given to "animal", since chlorpyrifos is an insecticide; the other food categories, except "detritus", were set to 0.
All the scores assigned are reported in Appendix D.

Procedure presented in section 8.2.2 was followed and the results are presented in table 8.4 and in figure 8.1.

Table 8.4. Sensitivity (*Se*), susceptibility to exposure (*Su*), recovery potential (*R*) and vulnerability (*V*) results for chlorpyrifos for each taxon considered in the microarthropod community. Results are ordered from highest to lower vulnerability values. Mites are reported in bold and springtails in italics.

TAXON	*Se*	*Su*	*R*	*V*
Hymenoptera (larvae)	12.5	16.7	5.0	34.8
Formicidae (larvae)	12.2	14.1	4.2	33.0
Hymenoptera	11.7	15.1	5.0	29.4
Diptera	14.5	11.7	4.8	29.1
Formicidae	11.7	12.7	4.2	28.4
Coleoptera (larve)	12.8	10.6	3.8	28.2
Diptera (larve)	12.5	11.9	4.8	25.6
Coleoptera	10.9	10.4	3.8	23.6
Lepidoptera (larvae)	12.7	11.4	5.3	22.9
Diplura	10.2	14.8	6.0	21.4
Chilopoda	10.0	14.4	5.8	21.2
Thysanoptera (nymphs)	11.0	11.3	5.0	20.6
Isotomidae	8.1	12.2	4.3	18.4
Prostigmata	7.3	14.6	5.0	17.8
Psocoptera	9.2	11.6	5.0	17.8
Diplopoda	9.5	11.0	5.0	17.4
Symphila	9.2	11.3	5.0	17.2
Thysanoptera	10.0	10.3	5.0	17.2
Neanuridae	8.1	12.6	5.0	16.9
Entomobryidae	8.1	12.3	5.0	16.6
Hypogastruridae	8.0	12.3	5.0	16.5
Araneae	6.2	14.0	4.4	16.0
Pauropoda	8.0	13.4	5.8	15.8
Mesostigmata	6.1	14.6	5.0	15.0
Onychiuridae	8.1	11.0	5.0	14.8
Tullbergiidae	8.0	10.9	5.0	14.6
Pseudoscorpionida	7.2	14.0	6.0	14.5
Hemiptera (larvae)	7.1	12.0	5.0	14.2
Protura	7.2	11.7	5.0	14.1
Sminthuridae	6.6	12.8	5.0	14.0
Katiannidae	6.0	12.9	5.0	13.0
Sminthurididae	6.0	12.7	5.0	12.7
Bourletiellidae	6.5	11.5	5.0	12.5
Hemiptera	7.1	10.0	5.0	11.9
Cryptostigmata	6.0	10.0	4.3	11.3
Astigmata	5.0	13.7	5.0	11.3
Neelidae	6.0	10.9	5.0	10.9
Isopoda	5.3	9.3	6.0	7.1

All the three components, of course, play a role in defining vulnerability. Anyway, it can be noted that in the top ten of more vulnerable taxa the first three (larvae of Hymenoptera, larvae of Formicidae and Hymenoptera) and

Diplura have the highest results for the susceptibility of exposure, higher than for sensitivity. Also adult ants have *Su* value higher than *Se*. All these taxa, indeed, live in the first soil layers, do not move vertically and their diet comprises animals (for Diplura also detritus is included in diet). Also for others vulnerable taxa (Diptera, larvae and adults, Coleoptera, larvae and adults, and larvae of Lepidoptera) the same considerations can be done, but, on the other hand, sensitivity values are higher than *Su* results. For a high proportion of these taxa recovery potential has a low value (*R* influence on vulnerability is opposite compared to *Se* and *Su*).
On the other hand, in the group composed by the ten less vulnerable taxa, five springtail families, two mite groups, Protura, Hemiptera and Isopoda are found. For all of them sensitivity is relatively low and susceptibility to exposure has a medium/low value, while recovery potential is high, except for Cryptostigmata. Anyway, for this group the other two parameters are low. Overall, these taxa are edaphic (except Hemiptera), relatively small and globular (except Isopoda and Protura), with respiration not via tracheae or book lungs (except a small proportion of proturans), with animals seldom included in diet, without complete metamorphosis and with strategies against the adverse conditions.

As a second step, for each a.i., scores were assigned to trait categories, according to section 8.2.1. Basically, only scores for food preference and respiration type varied according to chemicals compared to the chlorpyrifos example. Scores are presented in Appendix D. In figures from 8.1 to 8.8 results of the calculation of *V* are presented. The graphs show the ordination of taxa from the more to the less vulnerable. Varying only scores for respiration type and food preferences, chemicals were grouped according to the score values assigned following the procedures described in section 8.2.2:

- fungicides with 3 for “detritus”, 2 for respiration types related to water and 0 for respiration types related to air (only copper oxychloride);
- herbicides with 3 for “detritus”, 2 for respiration types related to water and 0 for respiration types related to air (only glyphosate);
- fungicides with 3 for “detritus”, 1 for respiration types related to water and 1 for respiration types related to air (only sulfur);
- fungicides with 0 for “detritus”, 3 for respiration types related to water and 1 for respiration types related to air (copper sulphate, cyprodinil, dimethomorph, fludioxonil, folpet and iprovalicarb);
- insecticides with 0 for “detritus”, 3 for respiration types related to water and 1 for respiration types related to air (only thiamethoxam);
- fungicides with 0 for “detritus”, 2 for respiration types related to water and 2 for respiration types related to air (only mancozeb);
- herbicides with 0 for “detritus”, 2 for respiration types related to water and 2 for respiration types related to air (only oxadiazon).

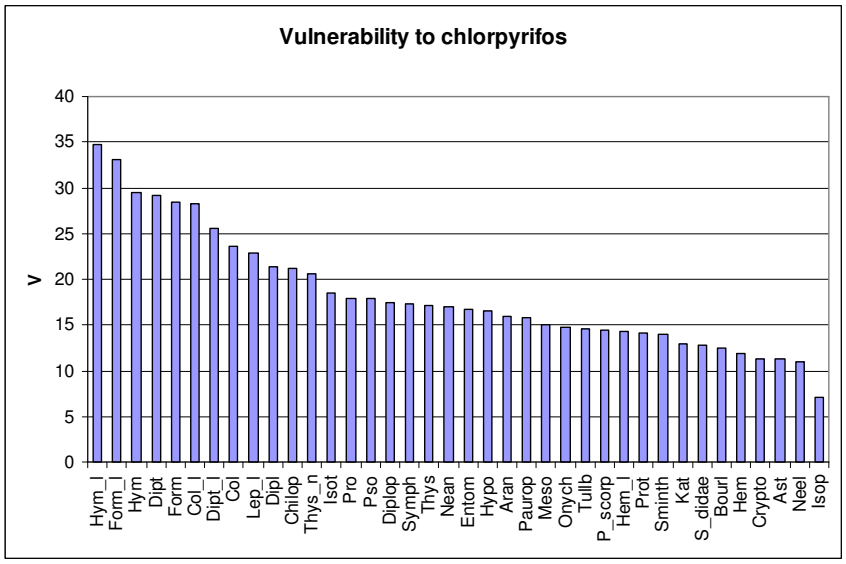

Figure 8.1. Vulnerability of different taxa to chlorpyrifos. Taxa abbreviations are reported in Appendix A.

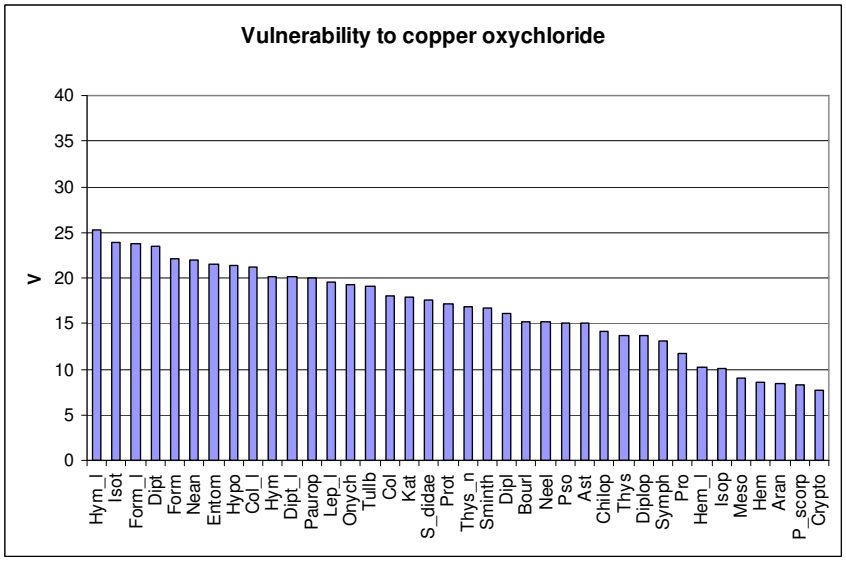

Figure 8.3. Vulnerability of different taxa to copper oxychloride. Taxa abbreviations are reported in Appendix A.

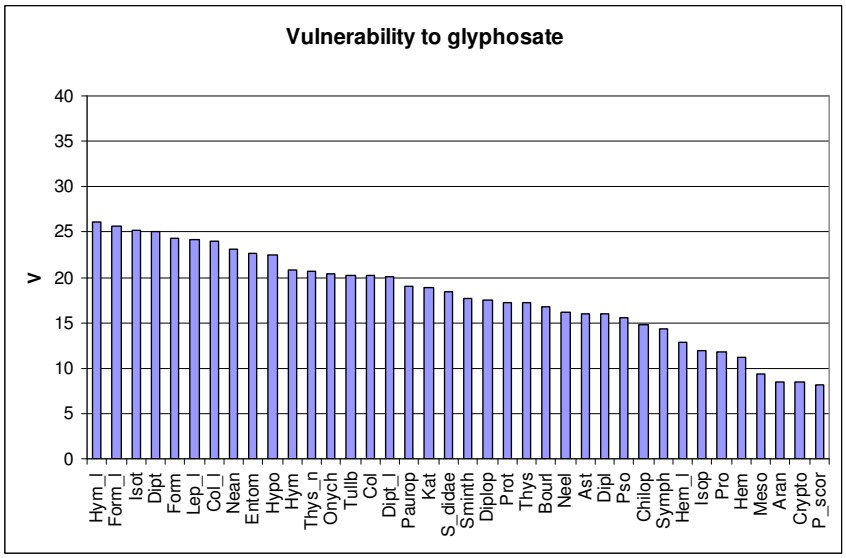

Figure 8.4. Vulnerability of different taxa to glyphosate. Taxa abbreviations are reported in Appendix A.

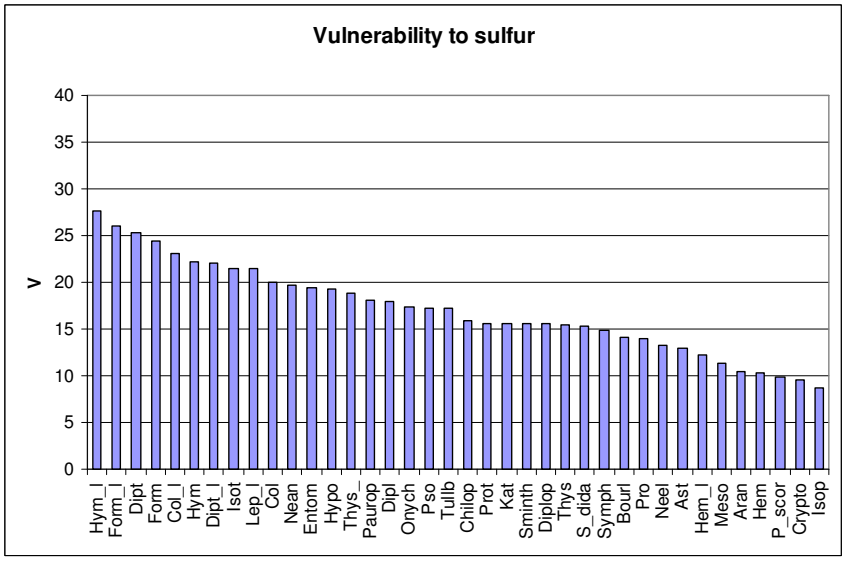

Figure 8.2. Vulnerability of different taxa to sulfur. Taxa abbreviations are reported in Appendix A.

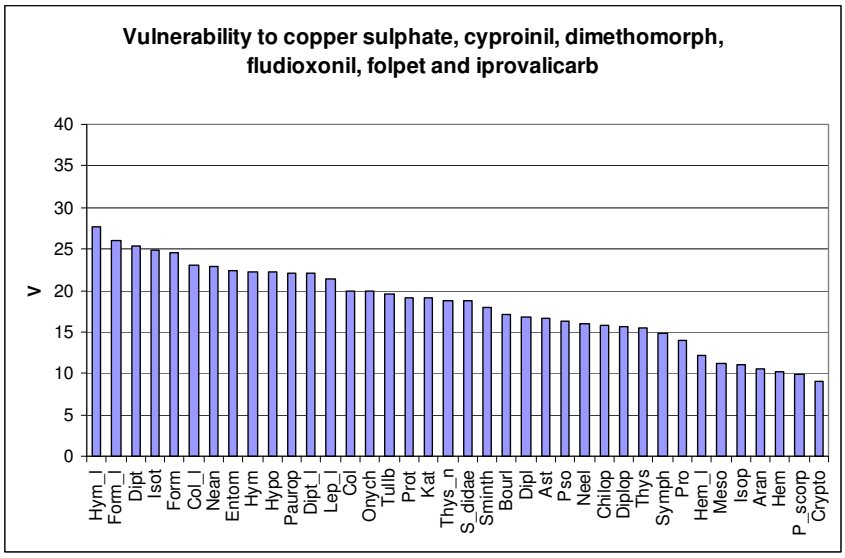

Figure 8.5. Vulnerability of different taxa to copper sulphate, cyprodinil, dimethomorph, fludioxonil, folpet and iprovalicarb. Taxa abbreviations are reported in Appendix A.

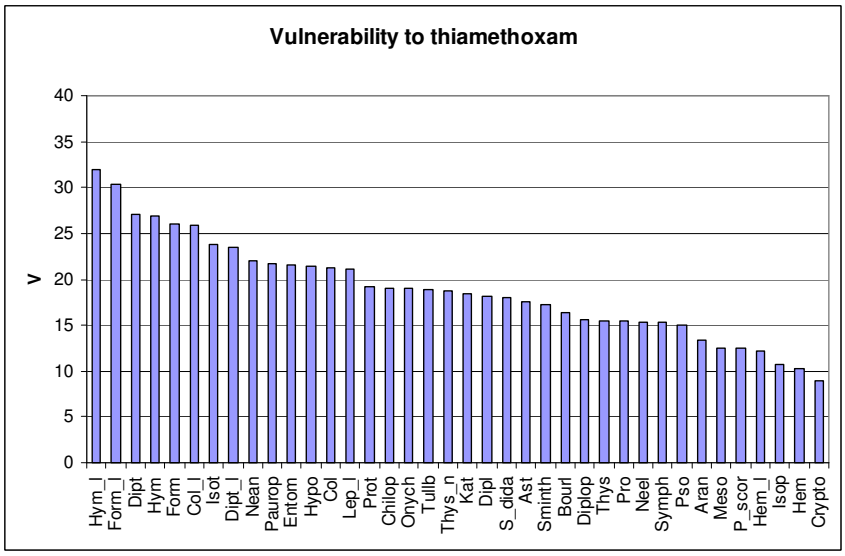

Figure 8.6. Vulnerability of different taxa to thiamethoxam. Taxa abbreviations are reported in Appendix A.

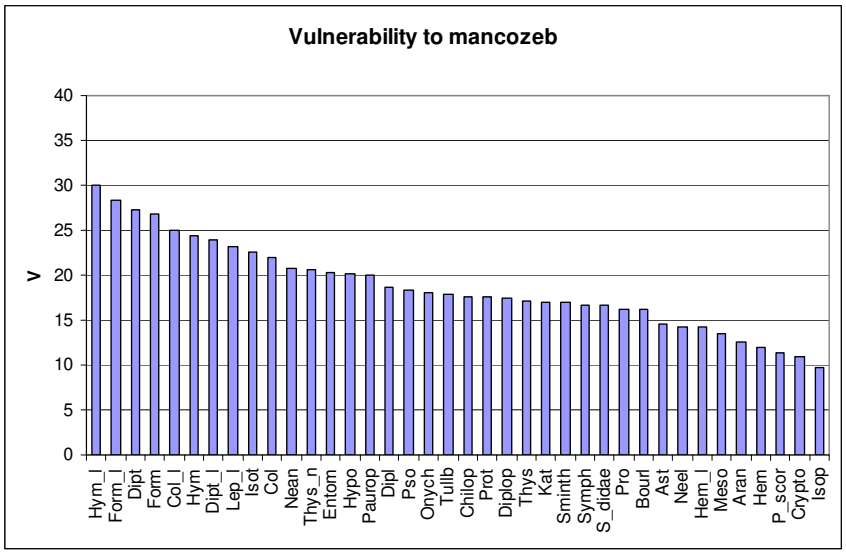

Figure 8.7. Vulnerability of different taxa to mancozeb. Taxa abbreviations are reported in Appendix A.

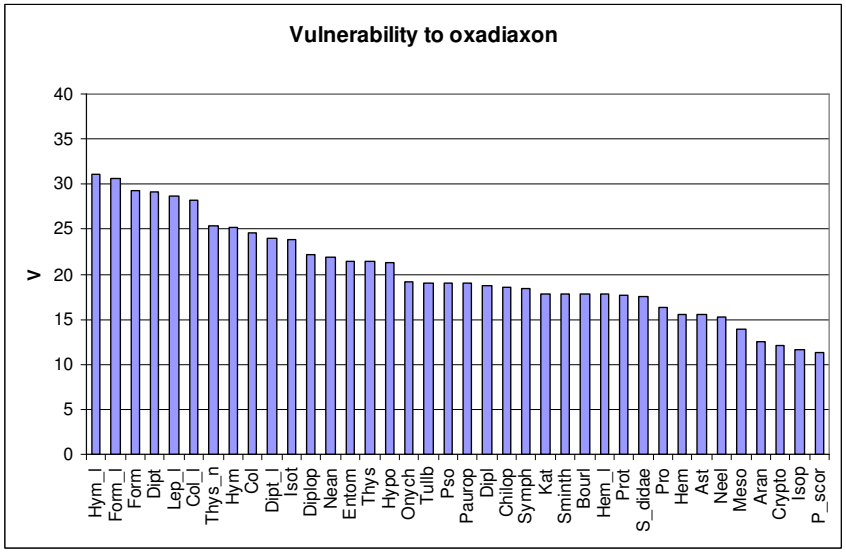

Figure 8.8. Vulnerability of different taxa to oxadiazon. Taxa abbreviations are reported in Appendix A.

Chlorpyrifos is the chemical for which vulnerability assumes highest values, especially for insects and insects larvae (first nine positions). This is a result

in accordance with its toxicity (insects are the target organisms), even if in the vulnerability analysis no toxicity data have been taken into account, but only traits related to toxicity.
Similarities and differences from the chlorpyrifos example previously shown are discussed.

- More vulnerable taxa.
Similarities with the chlorpyrifos example. Juveniles of Hymenoptera and, in particular, Formicidae, are the most vulnerable organisms for all the chemicals taken into account, although *V* assumes lower values for chemicals different from chlorpyrifos. Diptera, Formicidae and larvae of Coleoptera are always among the ten most vulnerable animals in the example. Hymenoptera were vulnerable to all the substances, but glyphosate. Diptera larvae were vulnerable also to thiamethoxam. Diplura showed high *V* values only for chlorpyrifos.
Differences with the chlorpyrifos example. Differences were driven by the traits related to respiration and food preferences, being the only traits varying among the different chemicals.
For mancozeb, sulfur and oxadiazon, the analysis considered as vulnerable the same taxa than chlorpyrifos, except Diplura.
Thysanoptera nymphs were vulnerable to oxadiazon, while the springtail family Isotomidae to mancozeb and sulfur. The diet is responsible to the inclusion of Thysanoptera nymphs among the more vulnerable taxa for a herbicide, since their diet comprise vegetation in a high proportion. Isotomidae were also vulnerable to all the other chemicals, except the mentioned chlorpyrifos and oxadiazon.
Other three families of Collembola (Neanuridae, Entomobryidae and Hypogastruridae) were considered as vulnerable for cyprodinil, dimethomorph, fludioxonil, folpet, glyphosate, iprovalicarb and copper (both oxychloride and sulphate). Neanuridae were vulnerable also to thiamethoxam. The differences regarding springtails between chlorpyrifos and other chemicals is mainly due to the fact that respiration with ventral tube was set to 0 for chlorpyrifos. Also oxadiazon did not comprise springtails among the most vulnerable taxa, because other organisms feeding on plant materials (Lepidoptera larvae, Coleoptera and Diptera larvae) had higher *V* values.
For the same reason (depletion of food source), Lepidoptera larvae were also vulnerable to the other herbicide, glyphosate.
Pauropoda showed high *V* values only for thiamethoxam, deriving from the cutaneous respiration and the diet comprising animals, which were set to 3 for this insecticide.

- Less vulnerable taxa.

Similarities with the chlorpyrifos example. The following taxa were comprised for all the chemicals: Isopoda, Hemiptera, and cryptostigmatid mites. Astigmatid mites were not vulnerable also to mancozeb, sulphur and oxadiazon, the springtail family Neelidae for mancozeb, sulfur, oxadiazon and thiamethoxam, Bourletiellidae to mancozeb, and Sminthurididae to oxadiazon.

Differences with the chlorpyrifos example. On the other hand, Pseudoscorpionida, Mesostigmata and Araneae were considered as not vulnerable for all the chemicals, but chlorpyrifos. Since they are predators, the cause could be the diet comprising animals. They are not vulnerable to the other insecticide, thiamethoxam, because for it the scores for a respiration type related to soil pore air are lower than chlorpyrifos.

Hemiptera larvae were not vulnerable for all the substances except oxadiazon. It is not surprising, being this taxon herbivore.

Prostigmatid mites were not vulnerable for all the chemicals but mancozeb and thiamethoxam. They breathe using tracheae and are predators, thus for the fungicide the cause could be the respiration type (score for tracheae: 2) and for the insecticide the diet (score for animals: 3).

Symphyla were not vulnerable to all the substances, except mancozeb, sulphur and oxadiazon. For mancozeb and oxadiazon it seems to be related to the respiration type, while for sulphur the cause could be the relative lower *V* value resulted for other taxa.

Thysanoptera were considered as not vulnerable, except for mancozeb, oxadiazon, sulfur, thiamethoxam and glyphosate. For mancozeb and oxadiazon the cause seems to be the score as high as 2 given for the respiration through tracheae, while for the herbicides the diet comprising mainly plant materials. Especially, for oxadiazon *V* value for this taxon resulted to be as medium/high, for the combination of the two causes. For sulfur and thiamethoxam the cause could be the relative less vulnerability of other taxa.

Psocoptera were not vulnerable to thiamethoxam (score 0 for the main item of their diet, plants, and score 1 for their respiration type, tracheae).

Chilopoda were not vulnerable to glyphosate (score 0 for both the main item of the diet, animals, and the respiration type, tracheae).

Although vulnerability does not comprise only sensitivity, a comparison with toxicity data would be a good tool for testing the achieved results. Unfortunately, toxicity data for soil microarthropods are scarce (not available or available with different endpoints, for a few chemicals and for very few organisms). For this reason the results were not compared to any toxicity data. Anyway, the aim of the chapter was to show an example in the application of the vulnerability concept to a natural community, more than giving a precise result in term of vulnerability for a specific taxon.

8.3.2. Vulnerability to a mixture

Following the procedures described in section 8.2.3, vulnerability to mixture was calculated (table 8.5).

Table 8.5. Vulnerability to mixture calculated using all the identified traits for each taxon, ordered from the more to the less vulnerable. Mites are reported in bold and springtails in italics.

TAXON	V_{mix}	TAXON	V_{mix}
Hymenoptera (larvae)	27.5	*Katiannidae*	16.3
Formicidae (larvae)	26.0	Protura	16.2
Diptera	25.3	*Sminthuridae*	16.0
Formicidae	24.4	*Sminthurididae*	16.0
Coleoptera (larve)	23.1	Chilopoda	15.8
Isotomidae	22.2	Diplopoda	15.7
Hymenoptera	22.1	Thysanoptera	15.5
Diptera (larve)	21.8	Symphyla	14.7
Lepidoptera (larvae)	21.5	*Bourletiellidae*	14.7
Neanuridae	20.4	*Neelidae*	13.8
Entomobryidae	20.0	**Prostigmata**	13.8
Coleoptera	20.0	**Astigmata**	13.6
Hypogastruridae	19.8	Hemiptera (larvae)	12.2
Thysanoptera (nymphs)	18.8	**Mesostigmata**	11.0
Pauropoda	18.6	Araneae	10.3
Onychiuridae	17.9	Hemiptera	10.3
Tullbergiidae	17.7	Pseudoscorpionida	9.6
Diplura	17.7	**Cryptostigmata**	9.4
Psocoptera	16.9	Isopoda	9.2

The order of taxa in terms of vulnerability is strongly influenced by the vulnerability to most present chemicals (i.e. the inorganic fungicides sulfur and copper oxychloride). The springtail family Neanuridae is the only not considered as among the most vulnerable to sulfur, but it has a high *V* value for copper oxychloride. The main differences with the chlorpyrifos example are Coleoptera and Diplura not considered as vulnerable to the mixture and Bourletiellidae, Sminthurididae, Sminthuridae, Katiannidae and Protura not included among the taxa with lowest *V* values for the mixture. For a discussion on the traits driving vulnerability see sections 8.2.1 and 8.3.1.

The procedure proposed here is of course rough compared to a risk assessment. In traditional procedures the predicted environmental concentration (PEC), also expressed as TWA, is compared to an ecotoxicological endpoint (with the same units) with a ratio, for giving a result in terms of Toxic Units. Thus, not only the exposure drives the risk result, because if a chemical is present with high concentrations but has a

low toxicity on an organism, the risk for this organism is low. On the other hand, a very toxic compound may pose a high risk even at low concentrations.
In the presented procedure it was not possible to use *V* in the same way of the ecotoxicological endpoint. Firstly, in the traditional procedures, both PEC and toxicity are expressed with the same unit, and comparing them means assessing if the concentration of the chemical is equal or not to the concentration giving a specific effect. Here, vulnerability takes into account not only the effect, but many traits acting also on susceptibility to exposure and recovery potential. Thus multiplying *V* for the proportion of the chemical in the compartment is a way to make a weighted mean of the *V* results for each substance.

8.3.3. Comparison with trait-based approach results

Vulnerability assessed in this chapter was compared with the result obtained with the trait-based analysis performed in chapter VII. Trait-based analysis considered as more vulnerable the following taxa: Diptera and their larvae, Coleoptera and their larvae, Hymenoptera and Lepidoptera larvae. Not vulnerable were Pauropoda, Protura and Diplura. Focusing on springtails most vulnerable were Sminthuridae and Bourletiellidae, while not vulnerable were Neanuridae, Tullbergiidae and, partly, Entomobryidae and Isotomidae. For mites Cryptostigmata and, partly, Mesostigmata groups were considered as not vulnerable.
The results obtained in this vulnerability analysis (table 8.5) are in accordance with those found in chapter VII referring to all the most vulnerable taxa of insects. Indeed, also in this analysis they are among the twelve most vulnerable, although the top of the ranking is not covered by one of them.
On the other hand, results are a bit controversial for Protura, Diplura and Pauropoda (intermediate vulnerability in the analysis performed in this chapter).
Results for Collembola are completely in disagreement: the families considered as vulnerable in the trait-based analysis are among the less vulnerable springtails in this chapter, and vice versa.
On the other hand, mites showed a good agreement, being Cryptostigmata and Mesostigmata the less vulnerable mites in both the analyses.
As highlighted in chapter VII, few traits may be responsible to the behaviour and the vulnerability of the community. An attempt of calculate vulnerability by using only the traits highlighted in chapter VII (exoskeleton/cuticle, respiration type, instars, diapause/supercooling and vertical movements) was made. Anyway, the new result was not satisfactory, for what concern the vulnerable taxa. The ordination for springtails did not change, being in

contrast with what observed in the trait-based analysis. It can be concluded that the reason for the differences between the two approaches should be general and not related to the so-called driving forces found in chapter VII.
The reasons for these differences can be identified and are listed below.

1. Trait based analysis has been based on field data, that are more complex compared to other data (e.g. laboratory results); moreover, soil is a highly variable compartment, thus the averages among replicates of the same sample were inevitably high.
2. Vulnerability analysis was based on literature information, not linked to the abundances found in a field situation, thus the comparison between the two analyses is biased by this factor.
3. Vulnerability analysis took into account a real situation, but in a determinate moment, while for trait-based analysis the behaviour of the community throughout the year was used.
4. As highlighted before, in both analyses only some traits were used, shaping the matrix with the availability of the information, since when data were missing for a trait, the whole trait was excluded from the procedures. This procedure may have led to the exclusion of some important traits.

8.4. Conclusions

A procedure for assessing ecological vulnerability for a microarthropod community was proposed and applied, referring to a real field situation as an example. Firstly, it was possible to identify the taxa more or less vulnerable to single chemicals. The results show small differences among substances because, as previously highlighted, vulnerability is chemical specific, at lest in relation to some traits. Then the vulnerability to the mixture was assessed, as a function of the exposure to chemicals. The result is influenced by the vulnerability to the chemicals most present in soil. Four insect taxa and their larvae (Hymenoptera, Formicidae, Diptera and Lepidoptera) were among the organisms more vulnerable to the mixture. They are all insects, holometabolous, living in the upper part of soil and don't move vertically in the compartment. Many of them are juveniles forms, that are usually more sensitive than their adults. Also two springtail families (Isotomidae and Neanuridae) resulted as vulnerable to the mixture. Their body shape, absence of a cuticle, vertical stratification, reduced movements in the vertical direction and duration of life in soil could be possible causes of this result. On the other hand, Isopoda, all the mite groups, Pseudoscorpionida, Hemiptera and their larvae, Araneae and the springtail family Neelidae are considered as less vulnerable to the mixture. They are edaphic organisms, without complete metamorphosis, some of them live in deeper layers, some possess a hard cuticle, or are small. Anyway, the explanation of the results

cannot be done analysing traits one by one, because vulnerability depends on the combination of them, as the equation that was used for quantification shows (eq. 8.1).
The results were compared with the conclusions of the trait-based analysis performed on the same community and reported in chapter VII, but several differences were identified, especially in defining the less vulnerable taxa within the whole community. The results for springtails were all in disagreement. Anyway, trait-based analysis was based on the community behaviour throughout the year, while the vulnerability assessment used only trait data and was referred to a fixed moment. The missing knowledge on some attributes of these organisms is one of the major shortcomings when working on soil community. Anyway, the approach seem promising, although the complexity of the field situation plays a relevant role.
Comparisons with other studies on vulnerability of soil community are not possible, because the application of this concept in ecotoxicology is relatively new and a very few studies on natural communities are available in the literature (for a review of the concept of vulnerability see De Lange *et al.*, 2010).
The aim of the chapter was to show a possible procedure of the application of the vulnerability concept to a natural community, not to give a precise quantification of the vulnerability of the organisms. The more ecological vulnerability analysis will be performed, the more they will produce the information for a sound basis in order to include this approach in the risk assessment procedures.

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References

Calow P, 1998. Ecological risk assessment: risk for what? How do we decide? *Ecotox Environ Saf* 40: 15-18.
Chapman PM, 2002. Integrating toxicology and ecology: putting the “eco” into ecotoxicology. *Mar Pollut Bull* 44:7-15.
Clendening LD, Jury WA, Ernst FF, 1990. A field mass balance study of pesticide volatilisation, leaching and persistence. In: DA Kurtz (Ed.), *Long Range Transport of Pesticides*. Lewis, Chelsea (MI), USA, pp. 47-60.
De Lange HJ, Lahr J, Van der Pol JJC, Wessels Y, Faber JH, 2009. Ecological vulnerability in wildlife: an expert judgment and multicriteria

analysis tool using ecological traits to assess relative impact of pollutants. *Environ Toxicol Chem* 28: 2233-2240.
De Lange HJ, Sala S, Vighi M, Faber JH, 2010. Ecological vulnerability in risk assessment – a review and perspectives. *Sci Total Environ* 408: 3871-3879.
EC (European Commission), 2003. *Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC for the new notified substances and the Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC on biocides*. Second ed. European Commission, Luxembourg, Luxembourg, Part 2, 328 pp.
FOOTPRINT website, 2006. The FOOTPRINT Pesticide Properties DataBase. Database collated by the University of Hertfordshire as part of the EU-funded FOOTPRINT project (FP6-SSP-022704). http://www.eu-footprint.org/ppdb.html (accessed in December 2010).
Ippolito A, Sala S, Faber JH, Vighi M, 2010. Ecological vulnerability analysis: a river basin case study. *Sci Total Environ* 408: 3880-3890.
Jury WA, Spencer WF, Farmer WJ, 1984. Behavior assessment model for trace organics in soil: III. Application of screening model. *J Environ Qual* 13: 573-579.
Mackay D, 2001. *Multimedia environmental models. The fugacity approach (second edition)*. Lewis publisher, CRC press LLC, Boca Raton (FL), USA, 261 pp.
Tomlin, CDS (Ed). *The pesticide manual: a world compendium (13th ed.)*. British Crop Protection Council, Farnham, UK, 1606 pp.
Van den Brink PJ, 2008. Ecological risk assessment: from book-keeping to chemical stress ecology. *Environ Sci Technol* 42:8999-9004.
Van Straalen NM, 1994. Biodiversity in ecotoxicological responses in animals. *Neth J Zool* 44:112-129.
Van Straalen NM, 2003. Ecotoxicology becomes stress ecology. *Environ Sci Technol* 37: 325-330A.
Verro R, Finizio A, Otto S, Vighi M, 2009. Predicting pesticide environmental risk in intensive agricultural areas. I: screening level risk assessment of individual chemicals in surface waters. *Environ Sci Technol* 43: 522-529.
Vighi M, Finizio A, Villa S, 2006. The Evolution of the Environmental Quality Concept: From the US EPA Red Book to the European Water Framework Directive. *Environ Sci Pollut R* 13: 9-14.

CHAPTER IX

General conclusions

The aim of the work was to improve the understanding of the actual consequences of a stressor for natural communities, both aquatic and terrestrial. For doing so, pesticides were identified as chemical stressors, traditional risk assessment methods were applied and the results were compared with effects observed on natural communities in field or with the applications of new tools.
In the aquatic environment risk due to the load of plant protection products (PPP) in a small river basin was characterised and the result was a high risk for algae, *Daphnia* and fish. Environmental samples from the basin (water and sediment) and from a reference river were used by Langer-Jaesrich and Scheil, (personal communication) as matrices for traditional ecotoxicological tests, improving realism (and complexity) related to the uncontrolled conditions of the matrix. No changes in the complexity related on community were made, i.e. only one species per test was used. Results were not in accordance with the high risk assessed in the environment. On the other hand, previous studies on macrozoobenthos communities performed in the same experimental areas by Bonzini *et al.* (2008) showed significant differences between the two rivers and the results were related to PPP load. Two main conclusions can be made from this part of the work. The risk assessment procedures overestimates risk for sake of protection, anyway for understanding the real consequences on communities approaches more complex than traditional tests on single species have to be used. The complexity in the tests should be put in the biotic part, not the abiotic.

Site-specific risk assessment was also performed for the soil environment, using earthworms as effect indicators. This choice was obligate, because, being *Eisenia foetida* the indicator species commonly used in soil ecotoxicological tests, toxicological data on it were available for all the chemicals involved in the mixture. For other organisms, e.g. microarthropods as springtails or mites, toxicological information is seldom available, for a few chemical and with different endpoints. Where possible, the differences in sensitivity between earthworms and microarthropods were highlighted and the result was that the latter are orders of magnitude more sensitive than *E. foetida* to pesticides. The need to involve these organisms in the lower tiers of the soil risk assessment was highlighted in order to improve the realism of the procedure for what concern the effects.

In the epigean terrestrial compartment a procedure for exposure assessment developed by Barmaz (2009) and validated through experimental monitoring was reported. The main value of the procedure is to overcome the concept of Hazard Quotient (HQ) that seems a too rough exposure estimation. An outline on differences and similarity among the procedures on the three compartments was given.

As highlighted several times and reported in Vighi *et al.* (2006) the need to study communities in field conditions is felt. A monitoring on microarthropod communities was performed in a vineyard under application of PPPs. Chemical stress was more important than the physical one. Main trends of taxa were identified according to stressors. Differences in behaviour of different taxa were observed: some organisms were affected by pesticide applications, while others were favoured, as a result of both indirect effects (e.g. lack of predation or of competition) and a low vulnerability to the stressor. This result could not be obtained through the application of traditional methods, only field investigations or new tools applied with sound basis could have detected them.
Indirect effects seemed important, thus an attempt to identify them within the trophic web was performed. Although the field situation is very complex, some indications of indirect effects of food source depletion could be seen. Anyway, the direct effect of chlorpyrifos application seems more important than the indirect ones.

Finally, the two new tools in ecotoxicology, trait-based approach (Baird and Van den Brink, 2007; Baird *et al.*, 2008) and vulnerability analysis (De Lange *et al.*, 2009; 2010) were applied on the microarthropod community. The attributes of the taxa within the community were described and linked to the abundances found in field. Traits linked to high or low vulnerability to chemical stressors were found. On the basis of these attributes more and less vulnerable taxa were determined.
Vulnerability was also analysed through the application of an index modified from Ippolito *et al.* (2010) ad using the traits previously identified. Chemical stressors were identified, referring to the exposure assessment in the vineyard performed in chapter III, and a realistic mixture was taken into account. Results were compared with those found through the trait-based analysis. Several differences were identified. Firstly, trait-based approach was applied referring to real conditions, while vulnerability was theoretically assessed. Moreover, and more important, a problem was the availability of information on traits. A lot of missing data could have biased the results and important traits may not be taken into account just for this problem. Anyway, the two approaches seem promising and, since a few studies using them are reported in literature, the need of experience in this field is felt to consolidate the theoretical basis.

Concluding, the research contributes to highlight the issue of "ecological realism", that should be taken more into account in ecotoxicology. It could be done by introducing the interspecific relations in the targets of the effect characterisation, or improving the exposure assessment with new procedures. Furthermore, the importance of field studies for understanding the actual consequences of stressors is highlighted. Being impossible to perform field monitoring for each site for which risk has to be assessed, new tools have to be used. Again, ecological realism is improved by using trait-based approach and vulnerability analysis. This work links the three approaches together, highlighting shortcomings and values and contributing to create a sound basis for including them into risk assessment procedures.

References

Baird DJ and Van den Brink PJ, 2007. Using biological traits to predict species sensitivity to toxic substances. *Ecotox Environ Saf* 67: 296-301.

Baird DJ, Rubach MN, Van den Brink PJ, 2008. Trait-based ecological risk assessment (TERA): the new frontier? *Integrated Environmental Assessment and Management* 4: 2-3.

Barmaz S, 2009. *Plant protection product risk assessment: distribution and experimental validation in terrestrial ecosystems.* PhD thesis, Doctorate in Environmental Sciences (XXII cycle), Faculty of Mathematical, Physical and Natural Sciences, University of Milano – Bicocca, Milano, Italy, 113 pp. http://hdl.handle.net/10281/7503.

Bonzini S, Finizio A, Berra E, Forcella M, Parenti P, Vighi M, 2008. Effect of river pollution on the colonisation of artificial substrates by macrozoobenthos. *Aquat Toxicol* 89: 1-10.

De Lange HJ, Lahr J, Van der Pol JJC, Wessels Y, Faber JH, 2009. Ecological vulnerability in wildlife: an expert judgment and multicriteria analysis tool using ecological traits to assess relative impact of pollutants. *Environ Toxicol Chem* 28: 2233-2240.

De Lange HJ, Sala S, Vighi M, Faber JH, 2010. Ecological vulnerability in risk assessment – a review and perspectives. *Sci Total Environ* 408: 3871-3879.

Ippolito A, Sala S, Faber JH, Vighi M, 2010. Ecological vulnerability analysis: a river basin case study. *Sci Total Environ* 408: 3880-3890.

Vighi M, Finizio A, Villa S, 2006. The evolution of the environmental quality concept: from the US EPA Red Book to the European Water Framework Directive. *Environ Sci & Pollut Res* 13: 9-14.

Appendix A. Taxa abbreviations

Aran	Araneae
Ast	Acari: Astigmata (Acaridida)
Bourl	Collembola: Bourletiellidae (suborder Symphypleona)
Chilop	Chilopoda
Col	Coleoptera
Col_l	Coleoptera (larvae)
Crypto	Acari: Cryptostigmata (Oribatida)
Dipl	Diplura
Diplop	Diplopoda
Dipt	Diptera
Dipt_l	Diptera (larvae)
Entom	Entomobryidae (suborder Entomobryomorpha)
Form	Hymenoptera: Formicidae
Form_l	Hymenoptera: Formicidae (larvae)
Hem	Hemiptera
Hem_l	Hemiptera (larvae)
Hym	Hymenoptera (others, different from Formicidae)
Hym_l	Hymenoptera (larvae) (others, different from Formicidae)
Hypo	Collembola: Hypogastruridae (suborder Poduromorpha)
Isop	Isopoda
Isot	Collembola: Isotomidae (suborder Entomobryomorpha)
Kat	Collembola: Katiannidae (suborder Symphypleona)
Lep_l	Lepidoptera (larvae)
Meso	Acari: Mesostigmata (Gamasida)
Nean	Collembola: Neanuridae (suborder Poduromorpha)
Neel	Collembola: Neelidae (suborder Neelipleona)
Onych	Collembola: Onychiuridae (suborder Poduromorpha)
P_scorp	Pseudoscorpionida
Paurop	Pauropoda
Pro	Acari: Prostigmata (Actinedida and Tarsonemida)
Prot:	Protura
Pso	Psocoptera
S_didae	Collembola: Sminthurididae (suborder Symphypleona)
Sminth	Collembola: Sminthuridae (suborder Symphypleona)
Symph	Symphyla
Thys	Thysanoptera
Thys_n	Thysanoptera (nymphs)
Tullb	Collembola: Tullbergiidae (suborder Poduromorpha)

Appendix B. Trait matrix

	max body size (mm)	body shape			
TAXON	body_size	glob	oval_flat	elong	very_elong
Isopoda	20[a,b]		1[a]		
Pseudoscorpionida	7.5[b]	1[g]			
Araneae	10[b]	1[a]			
Astigmata	1.8[c]	1[h]			
Prostigmata	10[c]	1[h]			
Cryptostigmata	1.3[c]	1[b]			
Mesostigmata	2[c]	0.9[h]	0.1[h]		
Hemiptera	5[d]	1[a]			
Hemiptera (nymphs)	4[e]	1[g]			
Psocoptera	6[a,d]	1[a]			
Thysanoptera	2[b,d]			1[g]	
Thysanoptera (nymphs)	1[e]			1[g]	
Coleoptera	40[a]	0.333[a]	0.667[a]		
Coleoptera (larve)	50[f]			1[a]	
Lepidoptera (larvae)	40[g]			1[a]	
Hymenoptera	40[a]			1[a]	
Hymenoptera (larvae)	30[h]			1[g]	
Formicidae	40[a]			1[a]	
Formicidae (larvae)	10[h]			1[a]	
Diptera	30[i]			1[a]	
Diptera (larve)	30[f]			1[a]	
Protura	2[a,b,d,j]			1[a]	
Diplura	10[a,j]			1[a]	
Hypogastruridae	1.39[k]			1[h]	
Neanuridae	1.95[l]			1[h]	
Onychiuridae	2.17[l]			1[h]	
Tullbergiidae	1.19[l]			1[h]	
Isotomidae	1.65[m]			1[h]	
Entomobryidae	2.67[l]			1[h]	
Bourletiellidae	1.13[n]	1[h]			
Katiannidae	0.83[n]	1[h]			
Sminthuridae	2.13[n]	1[h]			
Sminthurididae	0.7[n]	1[h]			
Neelidae	0.55[n]	1[h]			
Diplopoda	30[b]				1[a]
Chilopoda	60[a,o]				1[a]
Pauropoda	2[a,o]				1[a]
Symphyla	6[b]				1[a]

Appendix B

TAXON	exoskeleton/cuticle			life span (maximum age)		
	hard	chitinous	soft	1-2 m	2-12 m	>12 m
Isopoda	1[a]					1[o]
Pseudoscorpionida		1[h]				1[o]
Araneae	1[p]					1[o]
Astigmata	0.1[q]	0.9[q]			1[h]	
Prostigmata		1[q]			1[h]	
Cryptostigmata	1[b,q,r]				1[h,u]	
Mesostigmata	1[b,q]				1[h]	
Hemiptera		1[a]			1[f]	
Hemiptera (nymphs)		1[f]			1[f]	
Psocoptera			1[s]		1[f]	
Thysanoptera		1[s]			1[f,v]	
Thysanoptera (nymphs)			1[f]		1[f]	
Coleoptera	1[a,d,p,j,s]				1[f]	
Coleoptera (larve)			1[s]			1[f]
Lepidoptera (larvae)			1[s]	0.5[d]	0.5[d]	
Hymenoptera		1[s]			0.8[f]	0.2[f]
Hymenoptera (larvae)			1[f]	0.8[f]	0.2[f]	
Formicidae		1[s]			0.8[f]	0.2[f]
Formicidae (larvae)			1[f]		0.8[f]	0.2[f]
Diptera			1[s]		1[f]	
Diptera (larve)			1[f]		1[f]	
Protura		1[h]			1[w]	
Diplura			1[t]		1[x]	
Hypogastruridae			1[j,s]		1[y,z]	
Neanuridae			1[j,s]		1[y,z]	
Onychiuridae			1[j,s]		1[y,z]	
Tullbergiidae			1[j,s]		1[y,z]	
Isotomidae			1[j,s]		1[y,z]	
Entomobryidae			1[j,s]		1[y,z]	
Bourletiellidae			1[j,s]		1[y,z]	
Katiannidae			1[j,s]		1[y,z]	
Sminthuridae			1[j,s]		1[y,z]	
Sminthurididae			1[j,s]	0.1[n]	0.9[y,z]	
Neelidae			1[j,s]		1[y,z]	
Diplopoda	1[a,o]					1[o]
Chilopoda		1[a]				1[f]
Pauropoda			1[o]		1[f]	
Symphyla		1[h]			1[f]	

TAXON	n. generations per year		life stages (instars)	
	1 gen	more gen	holom	not_metam
Isopoda		1[f]		1
Pseudoscorpionida		1[o]		1
Araneae	0.8[f]	0.2[f]		1
Astigmata		1[f]		1
Prostigmata		1[f]		1
Cryptostigmata	0.333[r]	0.667[h,r]		1
Mesostigmata		1[f]		1
Hemiptera		1[aa]		1[j,cc]
Hemiptera (nymphs)		1[aa]		1[j,cc]
Psocoptera		1[d]		1[j,cc]
Thysanoptera		1[bb]		1[j,cc]
Thysanoptera (nymphs)		1[bb]		1[j,cc]
Coleoptera	1[f]		1[j,cc]	
Coleoptera (larve)	1[f]		1[j,cc]	
Lepidoptera (larvae)	0.25[d,cc]	0.75[d,cc]	1[j,cc]	
Hymenoptera		1[f]	1[j]	
Hymenoptera (larvae)		1[f]	1[j]	
Formicidae		1[f]	1[j]	
Formicidae (larvae)		1[f]	1[j]	
Diptera	0.5[d]	0.5[d]	1[j,cc]	
Diptera (larve)	0.5[d]	0.5[d]	1[j,cc]	
Protura	0.5[x]	0.5[f]		1[j]
Diplura		1[f]		1[j]
Hypogastruridae		1[cc]		1[j]
Neanuridae		1[cc]		1[j]
Onychiuridae		1[cc]		1[j]
Tullbergiidae		1[cc]		1[j]
Isotomidae	0.333[m]	0.667[m]		1[j]
Entomobryidae		1[cc]		1[j]
Bourletiellidae		1[cc]		1[j]
Katiannidae		1[cc]		1[j]
Sminthuridae		1[cc]		1[j]
Sminthurididae		1[cc]		1[j]
Neelidae		1[cc]		1[j]
Diplopoda		1[f]		1
Chilopoda		1[f]		1[o]
Pauropoda		1[f]		1
Symphyla		1[f]		1

Appendix B

TAXON	method of reproduction		duration of life in soil	
	bisex	parthenog	partly_in_soil	whole_in_soil
Isopoda	1[(f)]			1
Pseudoscorpionida	1[(f)]			1
Araneae	1[(f)]			1
Astigmata	0.5[(q)]	0.5[(q)]		1[(h,ff)]
Prostigmata	0.5[(q)]	0.5[(q)]		1[(h,ff)]
Cryptostigmata	0.5[(q)]	0.5[(q)]		1[(h,ff)]
Mesostigmata	0.5[(q)]	0.5[(q)]		1[(h,ff)]
Hemiptera	0.5[(j,cc)]	0.5[(j,cc)]	1	
Hemiptera (nymphs)	0.5[(j,cc)]	0.5[(j,cc)]		1
Psocoptera	0.5[(j,t,cc)]	0.5[(j,t,cc)]	0.5	0.5
Thysanoptera	0.5[(j)]	0.5[(j)]	1	
Thysanoptera (nymphs)	0.5[(j)]	0.5[(j)]		1
Coleoptera	0.9[(cc)]	0.1[(cc)]	0.5	0.5
Coleoptera (larve)	0.9[(cc)]	0.1[(cc)]		1
Lepidoptera (larvae)	0.9[(cc)]	0.1[(cc)]	0.5	0.5
Hymenoptera	0.5[(d,cc)]	0.5[(d,cc)]	0.5	0.5
Hymenoptera (larvae)	0.5[(d,cc)]	0.5[(d,cc)]		1
Formicidae	0.1[(d,f,cc)]	0.9[(d,f,cc)]		1
Formicidae (larvae)	0.1[(d,f,cc)]	0.9[(d,f,cc)]		1
Diptera	0.9[(cc)]	0.1[(cc)]	0.8	0.2
Diptera (larve)	0.9[(cc)]	0.1[(cc)]		1
Protura	1[(t)]			1
Diplura	1[(t)]			1
Hypogastruridae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Neanuridae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Onychiuridae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Tullbergiidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Isotomidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Entomobryidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Bourletiellidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Katiannidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Sminthuridae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Sminthurididae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Neelidae	0.5[(r,dd)]	0.5[(r,dd)]		1[(r,gg)]
Diplopoda	0.5[(o)]	0.5[(o)]		1
Chilopoda	0.9[(ee)]	0.1[(ee)]		1
Pauropoda	0.9[(ee)]	0.1[(ee)]		1
Symphyla	0.5[(o)]	0.5[(o)]		1

TAXON	respiration type				
	tracheae	v_tube	book_lungs	pleopods	cutan
Isopoda				1[(h)]	
Pseudoscorpionida			1[(h,o)]		
Araneae			1[(h,o)]		
Astigmata					1[(c,q)]
Prostigmata	1[(q)]				
Cryptostigmata	1[(q)]				
Mesostigmata	1[(q)]				
Hemiptera	1[(d)]				
Hemiptera (nymphs)	1[(e)]				
Psocoptera	1[(d)]				
Thysanoptera	1[(d)]				
Thysanoptera (nymphs)	1[(e)]				
Coleoptera	1[(d)]				
Coleoptera (larve)	1[(e)]				
Lepidoptera (larvae)	1[(j)]				
Hymenoptera	1[(d)]				
Hymenoptera (larvae)	1[(e)]				
Formicidae	1[(d)]				
Formicidae (larvae)	1[(e)]				
Diptera	1[(d)]				
Diptera (larve)	1[(cc)]				
Protura	0.1[(s,ee)]				0.9[(h,j,s,ee)]
Diplura	1[(j,s)]				
Hypogastruridae		0.67[(h,p)]			0.33[(h,j,p)]
Neanuridae		0.67[(h,p)]			0.33[(h,j,p)]
Onychiuridae		0.67[(h,p)]			0.33[(h,j,p)]
Tullbergiidae		0.67[(h,p)]			0.33[(h,j,p)]
Isotomidae		0.67[(h,p)]			0.33[(h,j,p)]
Entomobryidae		0.67[(h,p)]			0.33[(h,j,p)]
Bourletiellidae	0.25[(n)]	0.5[(h,p)]			0.25[(h,j,p)]
Katiannidae		0.67[(h,p)]			0.33[(h,j,p)]
Sminthuridae	0.25[(h,n)]	0.5[(h,p)]			0.25[(h,j,p)]
Sminthurididae		0.67[(h,p)]			0.33[(h,j,p)]
Neelidae		0.67[(h,p)]			0.33[(h,j,p)]
Diplopoda	1[(f)]				
Chilopoda	1[(o)]				
Pauropoda					1[(f)]
Symphyla	1[(o)]				

Appendix B

TAXON	type of food					
	bacteria	fungi	sapr/necr	detritus	plants	animals
Isopoda		0.1[b]	0.1[b]	0.1[b]	0.7[a,b]	
Pseudoscorpionida						1[b,o]
Araneae						1[b]
Astigmata		0.111[q]		0.167[q]	0.389[q]	0.333[q]
Prostigmata		0.188[q]			0.188[q]	0.625[q]
Cryptostigmata	0.056[q]	0.056[q]	0.333[q]	0.167[q]	0.389[q]	
Mesostigmata		0.125[q]			0.25[q]	0.625[q]
Hemiptera					1[d]	
Hemiptera (nymphs)					1[h]	
Psocoptera		0.321[d]		0.214[d]	0.464[d]	
Thysanoptera		0.05[d]			0.9[d]	0.05[d]
Thysanoptera (nymphs)		0.05[h]			0.9[h]	0.05[h]
Coleoptera		0.033[d]	0.268[d]	0.021[d]	0.420[a,d]	0.259[d]
Coleoptera (larve)		0.012[d]	0.151[d]	0.012[d]	0.420[a,d]	0.404[d]
Lepidoptera (larvae)		0.053[d]			0.947[d]	
Hymenoptera					0.133[d]	0.867[d]
Hymenoptera (larvae)		0.028[d]			0.194[d]	0.778[d]
Formicidae		0.125[b]			0.5[d]	0.375[d]
Formicidae (larvae)					0.333[d]	0.667[d]
Diptera			0.5[d]		0.25[d]	0.25[d]
Diptera (larve)		0.125[b]	0.375[d]		0.125[d]	0.375[b,d]
Protura		0.333[a]			0.333[a,d]	0.333[b]
Diplura		0.042[b]	0.25[r]	0.292[a]	0.042[b]	0.375[b,r]
Hypogastruridae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Neanuridae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Onychiuridae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Tullbergiidae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Isotomidae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Entomobryidae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Bourletiellidae		0.2[n]	0.2[n]		0.6[n]	
Katiannidae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Sminthuridae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Sminthurididae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Neelidae		0.231[b]		0.231[b]	0.462[b]	0.077[b]
Diplopoda					1[b]	
Chilopoda					0.2[b]	0.8[b]
Pauropoda	0.143[b]	0.393[b,r]			0.143[b]	0.321[b,r]
Symphyla	0.125[b]	0.125[b]			0.5[b,r]	0.25[r]

TAXON	vertical stratification				vertical movements	
	above	epi-	hemi-	eu-	vert_mov	no_vert_mov
Isopoda		1[a,gg]			1[b]	
Pseudoscorpionida		1[a]			1[f]	
Araneae		1[a]			1[f]	
Astigmata		0.5[f]	0.5[f]			1[f]
Prostigmata		0.5[f]	0.5[f]			1[f]
Cryptostigmata		0.5[f]	0.5[f]		1[b]	
Mesostigmata		0.5[f]	0.5[f]			1[f]
Hemiptera	0.5[f]	0.5[f]				1[f]
Hemiptera (nymphs)	0.5[f]	0.5[f]				1[f]
Psocoptera	1[a]					1[f]
Thysanoptera	1[d]					1[f]
Thysanoptera (nymphs)			1[f]			1[e]
Coleoptera	0.5[d]	0.5[gg]			1[f]	
Coleoptera (larve)		0.5[d]	0.5[d]		1[f]	
Lepidoptera (larvae)		0.9[cc]	0.1[cc]			1[f]
Hymenoptera	0.5[a]	0.5[f]				1[f]
Hymenoptera (larvae)		1[f]				1[f]
Formicidae		1[a]			0.5[f]	0.5[f]
Formicidae (larvae)		1[f]			0.5[f]	0.5[f]
Diptera	1[hh]					1[f]
Diptera (larve)		1[f]			1[f]	
Protura				1[b,ii]		1[f]
Diplura		1[b]				1[f]
Hypogastruridae		0.39[k]	0.45[k]	0.15[k]		1[b]
Neanuridae		0.75[jj]	0.17[jj]	0.08[jj]		1[b]
Onychiuridae		0.03[l]		0.97[l]		1[b]
Tullbergiidae				1[l]		1[b]
Isotomidae		0.48[m]	0.32[m]	0.2[m]		1[b]
Entomobryidae		0.58[jj]	0.22[jj]	0.2[jj]		1[b]
Bourletiellidae		0.5[n]	0.5[n]			1[b]
Katiannidae		1[n]				1[b]
Sminthuridae		0.86[n]	0.14[n]			1[b]
Sminthurididae		0.67[n]	0.33[n]			1[b]
Neelidae				1[n]		1[b]
Diplopoda		1[a]				1[f]
Chilopoda		1[a]			0.5[a]	0.5[a]
Pauropoda		1[a]				1[a]
Symphyla		1[a]			1[a]	

Appendix B

TAXON	strategies against adverse conditions		position in trophic web	
	diap/s.cool	no_diap/s.cool	1st_cons	2nd_cons
Isopoda	1[(a)]		1[(ll)]	
Pseudoscorpionida	1[(b)]			1[(ll)]
Araneae	1[(f)]			1[(ll)]
Astigmata	1[(jj)]		0.67[(ll)]	0.333[(ll)]
Prostigmata	1[(jj)]		0.375[(ll)]	0.625[(ll)]
Cryptostigmata	1[(jj)]		1[(ll)]	
Mesostigmata	1[(jj)]		0.375[(ll)]	0.625[(ll)]
Hemiptera	1[(f)]		1[(ll)]	
Hemiptera (nymphs)	1[(f)]		1[(ll)]	
Psocoptera	0.5[(kk)]	0.5[(kk)]	1[(ll)]	
Thysanoptera	0.5[(d)]	0.5[(d)]	0.95[(ll)]	0.05[(ll)]
Thysanoptera (nymphs)	0.5[(f)]	0.5[(f)]	0.95[(ll)]	0.05[(ll)]
Coleoptera	0.2[(d)]	0.8[(f)]	0.74[(ll)]	0.26[(ll)]
Coleoptera (larve)	1[(f)]		0.6[(ll)]	0.4[(ll)]
Lepidoptera (larvae)	1[(j)]		1[(ll)]	
Hymenoptera	1[(f)]		0.13[(ll)]	0.87[(ll)]
Hymenoptera (larvae)	1[(f)]		0.22[(ll)]	0.78[(ll)]
Formicidae	1[(f)]		0.625[(ll)]	0.375[(ll)]
Formicidae (larvae)	1[(f)]		0.33[(ll)]	0.67[(ll)]
Diptera		1[(f)]	0.75[(ll)]	0.25[(ll)]
Diptera (larve)	1[(f)]		0.625[(ll)]	0.375[(ll)]
Protura	1[(f)]		0.67[(ll)]	0.33[(ll)]
Diplura	1[(f)]		0.625[(ll)]	0.375[(ll)]
Hypogastruridae	1[(y)]		0.92[(ll)]	0.08[(ll)]
Neanuridae	1[(y)]		1[(ll)]	
Onychiuridae	1[(f,y)]		1[(ll)]	
Tullbergiidae	1[(y)]		1[(ll)]	
Isotomidae	1[(y)]		1[(ll)]	
Entomobryidae	1[(y)]		1[(ll)]	
Bourletiellidae	1[(n,y)]		1[(ll)]	
Katiannidae	1[(y)]		1[(ll)]	
Sminthuridae	1[(h,y)]		1[(ll)]	
Sminthurididae	1[(y)]		1[(ll)]	
Neelidae	1[(y)]		1[(ll)]	
Diplopoda	0.5[(a)]	0.5[(a)]	1[(ll)]	
Chilopoda	1[(f)]			1[(ll)]
Pauropoda	1[(f)]		0.80[(ll)]	0.2[(ll)]
Symphyla	1[(f)]		0.75[(ll)]	0.25[(ll)]

For trait categories abbreviations see Appendix C.

(a) Angelini P, Fenoglio S, Isaia M, Jacomini C, Migliorini M, Morisi A, 2002. *Tecniche di biomonitoraggio della qualità del suolo*. ARPA Piemonte, Torino, Italy, 106 pp. In Italian.
(b) Codurri M, Truzzi A, Bertonazzi MC, 2005. *Microartropodi del terreno – manuale da campo per il riconoscimento dei microartropodi del terreno come indicatori della qualità biologica del suolo (metodo QBS-ar)*. Consorzio del parco naturale dell'Oglio sud, Calvatone (CR), Italy, 104 pp. In Italian.
(c) Kranz GW, 1979. *A manual of acarology*. Oregon State University Book Stores, Inc., Corvallis (OR), USA, 509 pp.
(d) Chinery M, Pandolfi M (Ed), 1998. *Guida degli insetti d'Europa – atlante illustrato a colori*. Franco Muzzio Editore, Padova, Italy, 431 pp. In Italian.
(e) Value derived from adult forms.
(f) Masin S, personal communication.
(g) Direct observations.
(h) Van Gestel CAM and Berg M, personal communication.
(i) Istruzione agraria online. Website: www.agraria.org (accessed in Spetember 2010). In Italian.
(j) Gulla PJ, Cranston PS, 1994. *The insects: an outline of entomology*, Chapman & Hall, London, UK, 491 pp.
(k) Thibaud JM, Schulz HJ, da Gama Assalino MM, 2004. *Synopses on Palearctic Collembola Volume 4 HYPOGASTRURIDAE*, Staatliches Museum für naturkunde Görlitz, Görlitz, Germany, 287pp.
(l) Gisin H, 1960. *Collembolenfauna europea.* Hauptkonservator am Natur-historischen Museum Genf, Genève, Switzerland, 312 pp. In German.
(m) Jørgensen HB, Hedlund K, Axelsen JA, 2008. Life-history traits of soil collembolans in relation to food quality. *Appl Soil Ecol* 38: 146-151.
(n) Koolhas JE, Van Gestel CAM, Römbke J, Soares AMVM, Jones SE, 2004. Ring-testing and field-validation of a Terrestrial Model Ecosystem (TME) – an instrument for testing potentially harmful substances: effects of carbendazim on microarthropod communities. *Ecotoxicology* 13: 75-88.
(o) Barnes RD, Milani R (Ed.), 1985. *Zoologia: gli invertebrati*. Piccin Nuova Libraria, Padova, Italy, 1237 pp. In Italian.
(p) Wiles JA and Frampton GK, 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. *Pestic Sci* 47: 273-285.
(q) Ruf A and Beck L, 2005. The use of predatory soil mites in ecological soil classification and assessment concepts, with perspectives for oribatid mites. *Ecotox Environ Saf* 62: 290-299.
(r) Butcher JW, Snider R, Snider RJ, 1971. Bioecology of edaphic Collembola and Acarina. *Annu Rev Entomol* 16:249-288.
(s) Grandi G, 1966. *Istituzioni di entomologia generale*. Edizioni Calderini, Bologna, Italy, 654 pp. In Italian.
(t) Capinera JL (Ed.), 2008. *Encyclopedia of Entomology Second edition*, Springer, Dordrecht (The Netherlands), 4346 pp.
(u) Van Straalen NM and Van Gestel CAM, 1994. Soil invertebrates and micro-organisms, in: Calow (Ed.), *Handbook of Ecotoxicology vol.1*, Blackwell Scientific Publications, Oxford, UK, 478 pp.
(v) CBW Info. Website: www.cbwinfo.com/Biological/BWList.shtml (accessed in September 2010).

(w) Mean value or analogy with similar taxa.
(x) BayScience Foundation Inc., 2004-2009. ZipcodeZoo website: www.zipcodezoo.com/Key/Animalia/animalia_kingdom.asp (accessed in September 2010).
(y) Hopkin S, 1997. *Biology of the springtails (Insecta: Collembola)*. Oxford University Press, Oxford, UK, 334 pp.
(z) Earthlife Web. Website: www.earthlife.net/insects (accessed in Sptember 2010).
(aa) Kendall DA, 2010. Website: www.kendalluk.com (accessed in September 2010).
(bb) Agriculture and Natural Resources, University of California, 2010. UC IPM Online website: www.ipm.ucdavis.edu (accessed in September 2010).
(cc) Masutti L, Zangheri S, 2001. *Entomologia generale e applicata*, CEDAM, Padova, Italy, 978 pp. In Italian.
(dd) Siepel H, 1994. Life-history tactics of soil microarthropods. *Biol Fertil Soils* 18: 263-278.
(ee) Animal Life Resource, 2010. Website: animals.jrank.org (accessed in September 2010).
(ff) Büchs W, 1994. Effects of different input of pesticides and fertilizers in the abundance of arthropods in a sugar beet crop: an example for a long-term risk assessment, in: Donker MH, Eijsackers H, Heinbach F (Eds.), *Ecotoxicology of soil organisms*, Lewis Publisher, Boca Raton (FL), USA, 470 pp.
(gg) Van Straalen NM and Van Rijn JP, 1998. Ecotoxicological risk assessment of soil fauna recovery from pesticide application. *Rev Environ Contam Toxicol* 154: 83-141.
(hh) Gruppo SDG, Servizi generali disinfestazioni. Website: www.grupposgd.it/guida-infestanti (accessed in September 2010). In Italian.
(ii) Petersen H, Luxton M, 1982. A comparative analysis of soil fauna populations and their role in decomposition process. *OIKOS* 39: 287-388.
(jj) Bellinger PF, Christiansen KA, Janssens F, 1996-2010. Checklist of the Collembola of the World. Website: www.collembola.org (accessed from October 2008 to April 2010).
(kk) Insect Identification, 2009. Website: www.insectspedia.blogspot.com (accessed in September 2010).
(ll) Derived from “food preference” trait value.

Appendix C. Trait categories abbreviations

Body size
- body_size: maximum body length

Body shape
- glob: globular
- oval_flat: oval flattened
- elong: elongated
- very_elong: very elongated

Exoskeleton/cuticle
- hard: hard (calcareous cuticle, elytron or sclerified tegument)
- chitinous: chitinous (as arthropod exoskeleton)
- soft: soft body

Life span (maximum age)
- 1-2 m: 1-2 months
- 2-12 m: 2-12 months
- >12 m: more than 12 months

Number of generations per year:
- 1 gen: one generation per year
- more gen: more generations per year

Life stages (instars)
- holom: holometabolic (complete metamorphosis)
- not_metam: not complete metamorphosis

Method of reproduction
- bisex: bisexual
- parthenog: parthenogenetic

Duration of life in soil
- partly_in_soil: only part
- whole_in soil: whole life

Respiration type
- tracheae: tracheae
- v_tube: ventral tube
- book_lungs: book lungs
- pleopods: pleopods
- cutan: cutaneous

Appendix C

Food preference
- bacteria: bacteria
- fungi: fungi
- sapr/necr: dead organisms, excrements and structured detritus of animal origin.
- detritus: amorphous detritus
- plants: plant materials (leaves, roots, mycorrhizae, algae, structured vegetal detritus)
- animals: animals (for predators and parasites)

Vertical stratification
- above: aboveground
- epi-: epigeic
- hemi-: hemi-edaphic
- eu-: eu-edaphic

Vertical movements
- vert_mov: possibility of vertical movements
- no_vert_mov: no vertical movements

Strategies against adverse conditions (diapause, supercooling)
- diap/s.cool: presence of strategies against adverse conditions
- no_diap/s.cool: absence of strategies against adverse conditions

Position in trophic web
- 1st_cons: 1st consumers
- 2nd_cons: 2nd consumers

Appendix D. Scores assigned to traits for the quantification of vulnerability

D.1. Traits with changing values according to chemical

	sensitivity					*susceptibility to exposure*					
	respiration type					**type of food**					
Chemical	tracheahe	ventral tube	book lungs	pleopods	cutaneous	bacteria	fungi	sapr/necr	detritus	plants	animals
chlorpyrifos	2	0	2	0	0	0	0	0	3	0	3
copper oxychloride	0	2	0	2	2	0	3	0	3	0	0
copper sulphate	1	3	1	3	3	0	3	0	0	0	0
cyprodinil	1	3	1	3	3	0	3	0	0	0	0
dimethomorph	1	3	1	3	3	0	3	0	0	0	0
fludioxonil	1	3	1	3	3	0	3	0	0	0	0
folpet	1	3	1	3	3	0	3	0	0	0	0
glyphosate	0	2	0	2	2	0	0	0	3	3	0
iprovalicarb	1	3	1	3	3	0	3	0	0	0	0
mancozeb	2	2	2	2	2	0	3	0	0	0	0
oxadiazon	2	2	2	2	2	0	0	0	0	3	0
sulfur	1	1	1	1	1	0	3	0	3	0	0
thiamethoxam	1	3	1	3	3	0	0	0	0	0	3

Appendix D

D.2. Traits with not changing values according to chemical

	sensitivity				
	body length	**body shape**			
Chemical		globular	oval flattened	elongated	very elongated
All chemicals	*	1	2	3	3

*3 for body length < 2 mm; 2 for body length ≥ 2 mm and < 10 mm; 1 for body length ≥ 10 mm.

	sensitivity						
	exoskeleton/cuticle			**instars**		**strategies against adverse contitions**	
Chemical	hard	chitinous	soft	holometabolic	not metamorphosis	diapause / supercooling	no diapause / supercooling
All chemicals	1	2	3	3	1	1	3

	susceptibility to exposure								
	life span (maximum age)			**duration of life in soil**		**vertical stratification**			
Chemical	age 1-2 m	age 2-12 m	age0 >12 m	partly life in soil	whole life in soil	aboveground	epigeic	hemi-hedaphic	eu-edaphic
All chemicals	3	2	1	1	3	3	3	2	1

	susceptibility to exposure			
	vertical movements		**position in trophic web**	
Chemical	yes vertical movements	no vertical movements	1^{st} consumers	2^{nd} consumers
All chemicals	1	3	1	3

	recovery potential			
	n. generations per year		**method of reproduction**	
Chemical	1 generation per year	more generations per year	bisexual	parthenogenetic
All chemicals	1	3	3	1

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