Bioindicators for the assessment of side effects of pesticides on soil biological status: report of a collaborative study conducted for the french ministry of the environment

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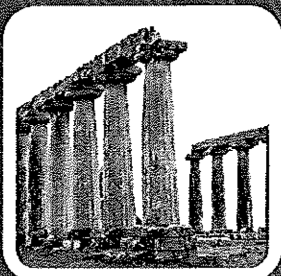
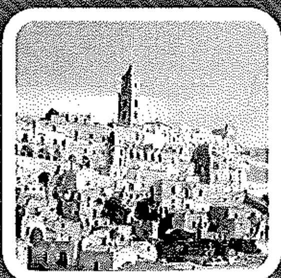
BOOK OF ABSTRACTS





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BIOINDICATORS FOR THE ASSESSMENT OF SIDE EFFECTS OF PESTICIDES ON SOIL BIOLOGICAL STATUS: REPORT OF A COLLABORATIVE STUDY CONDUCTED FOR THE FRENCH MINISTRY OF THE ENVIRONMENT

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SUMMARY

Soil is a limited natural resources allowing the primary production but also contributing to ecosystemic services such as C and N cycling. Although soil carries out functions that are crucial for the environment and life on earth, it is under increasing environmental pressure mostly due to the intensification of human activities, which are damaging the capacity of soil to continue to perform in full its broad variety of crucial functions. Methods to monitor soil functioning remain scarce and there is a need to develop tools to estimate the impact of agricultural practices, including pesticide treatment, on the sustainability of soil biological status. On behalf of French Ministry of Ecology a collaborative program of research was recently conducted to test existing methods as well as new and/or under development methods to estimate the impact of pesticides on soil microbial communities' abundance, diversity and activity with a focus on several functional communities. The speaker

will synthesize the results of this study and discuss it to define new perspectives of research to define bioindicators to assess the side effect of pesticides on soil biological status.

Keywords: Bioindicator, pesticide, soil biological status, ecotoxicological impact

INTRODUCTION

The recent revision of the EU water framework (EU directive 2000/60) defined new lines in which not only the quality of drinking water is considered (EU limit for drinking water 0,1 µg of pesticide per liter) but also the quality of water bodies, including rivers and lakes. In addition, the EU report of the working group established under "the thematic strategy soil protection" aiming at preparing the EU proposal concerning the soil framework, clearly identifies agriculture practices which in most of the cases rely on pesticide use, as a major threat for soil biodiversity and functioning. The Millennium Ecosystem Assessment, an international effort to inventory global ecosystems, also identified agriculture as a major threat for soil ecosystemic functions, notably C cycling which may indeed contribute to global change by destocking C from agricultural soil. Up to now there are quite few methods available to estimate the impact of agricultural practices, notably those resulting from pesticides application, onto soil microbiota functioning which plays a key role in geochemical cycles. Indeed in the dossier required for the authorization of pesticides (EU Directive 91/414) only three tests revealing the impact of pesticide application on global microbial parameters are mentioned and none of them provides an estimate of pesticide impact on soil microbial communities functioning. Therefore, there is an urgent need to develop and to test new methods to estimate the impact of pesticides on microbial communities which are responsible for the soil ecosystemic services. These services are of prime since disequilibrium in N or C cycles leads to greenhouse gas production N_20, NO or CO_2, respectively. Several reports suggest that agriculture is responsible for the liberation of C stored in the soil and for the transformation of nitrate, applied on the crop to promote their development, to N_2O and NO which contribute to greenhouse gas emission. In addition, the filter capability of the soil (biotransformation of pollutants) may contribute to decrease the persistence of pesticides in the different compartments of the environment. Therefore, the development of a sustainable agriculture must take into account the impact of agricultural practices on soil ecosystemic services.

Although, quite numerous studies are dealing with the evaluation of pesticides impact on soil microflora, the number of microbial tests available for operational perspective remains scarce. Indeed when considering the ISO catalog under the section 'Soil quality-Biological methods' only few methods are identified and listed thereafter: (i) biodegradation/mineralization of chemical substances under aerobic (ISO 11226, ISO 14239) or anaerobic (ISO 15473) conditions, (ii) estimation of the microbial abundance and activity (ISO 17155 et ISO 16072), estimation of the microbial biomass by substrate induced respiration (SIR) or fumigation-extraction (ISO 14240), determination of nitrogen mineralization and nitrification (ISO 14238), the estimation of pollutants on the germination of spores of endomycorrhizal fungi (experimental standards X31-205-1 et 2). In addition to these standards new standards are under development at the ISO level among which 'the Determination of soil microbial diversity by phospholipids fatty acid analysis (PLFA)' (ISO TC190/SC4/n397) and 'the direct extraction of nucleic acids from soil' (ISO CD 11063). The last standard opens new insight in term of development of innovative tools to estimate the impact of pesticides on microbial functional communities responsible for the key soil ecosystemic functions.

In this context, to fill this gap of knowledge, a collaborative scientific project was funded by the French Ministry of Ecology with the aim to test methods of reference as well as new methods (for some of them under development) to estimate the possible impact of pesticides applied in agriculture on soil functional microbial biodiversity.

EXPERIMENTAL DESIGN

In order to reach this objective, we have chosen to target vineyards on which pesticides are often applied. An experiment was conducted for two years in the vineyards of Bordeaux by comparing three agricultural practices (i) grass strip, (ii) mechanical weeding and (iii) chemical weeding. In addition, an experiment was conducted on microcosms incubated under laboratory conditions in order to control the exposure of soil microbial communities to different concentrations of pesticides. Several types of indicators were chosen and tested:

-(i) *global indicators*: microbial C biomass (Chaussod *et al.* 1999), FDA activity, microbial respiration, nitrogen potentially minerasable (Catroux *et al.* 1987), enumeration of living microbial cells (Pascault *et al.* 2009), deshydrogenase activity, biochemical diversity (BIOLOG®) (Garland and Mills 1991), specific diversity (T-RFLP) (Muyser *et al.* 1993),

(ii) *specific indicators:* (a) *AM fungal community*: germination of spores of AM fungi (AFNOR X31-205-1), root colonization by AM fungi (AFNOR X31-205-2), genetic diversity and abundance of AM fungi in soil (Gianinazzi *et al.* 2005, Weissenhorn and Leyval 1996); (b) *C cycling*: structure and

abundance of the bacterial community responsible for the degradation of the protocatechuate (Martin-Laurent *et al.* 2001; El Azhari *et al.* 2008); (c) *N cycling*: abundance and activity of the bacterial community responsible for nitrification (Okano *et al.* 2004); (d) *pesticide biodegradation*: abundance of the microbial populations involved in pesticides biodegradation; (e) *mineralization of organic sulphur:* abundance and activity of the microbial community responsible for the mineralization of organic sulfur (Tabatabaï M.A. and Bremner J.M. 1970, Tabatabaï 1984).
In addition, to this ecotoxicological data-set the exposure of the soil microbial community was estimated by measuring pesticide residues over the incubation period after their extraction from the soil matrix and their quantification by GC-MS.

RESULTS

Our collaborative project was aiming at assessing chosen bioindicators having the potential to describe the impact of pesticides on soil ecosystemic functions by measuring the abundance, activity and diversity of selected microbial communities taken at different levels of biological organisation: from the community to the gene. This project was carried out with the aim to identify bioassays which could routinely be used for regulation and/or environmental monitoring. The advantages of these tools have been compared to those of more conventional approaches.
Overall, it was found that a mixture of different pesticides commonly used in wine growing areas (folpel, deltamethrin and fenhexamid), applied at agronomical doses, had little or no effect on the global parameters often used to describe the soil microflora.
Different bioindicators, microbial biomass, FDA hydrolase activity, respiratory activity, potentially mineralizable N, number of viable bacterial cell, biochemical diversity (BIOLOG®), and dehydrogenase activity, were used to asses side-effects of pesticides. Only biochemical diversity and, in some cases, dehydrogenase activity were found to vary significantly in response to pesticides treatment or as a result of soil management practices (grass strip, chemical weeding and mechanical weeding).
Different tests conducted with the aim to describe arbuscular mycorrhyzal fungi (spore germination and colonisation potential) were found positive but not statistically significant. However, the estimation of the abundance and of the diversity of AM fungal populations from DNA directly extracted from soil seems to be promising.
Pesticides did not show appreciable effects on the abundance, activity and diversity of functional communities involved in carbon cycle (*pca*H commu-

nity), nitrogen cycle (*amoA* community) or organic sulphur mineralisation (ARS community) cycles. However, one could notice that at acute pesticide concentrations the abundance of the *pcaH* community was significantly decreased and dose-response curve could be established.
The physico-chemical characteristics of the chemicals tested here, high Koc (i.e. deltamethrin, folpel), and susceptibility to volatilisation (folpel) combined with a reduced DT50 (fenhexamid) contribute to a moderate exposure of the soil microbial community and may partly explain the rather low sensibility of the biodincators tested here.

CONCLUSIONS

This study highlights the difficulties often encountered for estimating the impact of pesticides on soil biological status. Here, we have chosen to use biochemical, microbiological and molecular tools targeting indigenous soil microflora to estimate the impact of its exposure to a pesticide cocktail representative of the practices applied in the Bordeaux vineyard. We showed that the methods of reference (microbial C biomass, potentially minerasable N,...) were sensitive enough to estimate the impact of different weeding practices strategies (grass strip, mechanical weeding and chemical weeding) but not to estimate the impact of pesticide treatments (at least not from the statistical point of view). In addition, the new methods tested here notably those based on direct soil DNA extraction and further analysis by PCR-based approaches targeting different functional groups did not allow measuring the impact of pesticides on the targeted functions. Among the functional groups targeted, the AM fungal community seems to be promising at least for estimating the impact of weeding strategies and to a lesser extent to estimate the counter side effect of fungicide application. This study showed the limitations of studying functional groups and suggests that, if of interest, these groups, which often showed an important functional redundancy, should be studied with tools targeting more labile compounds such as mRNA which showed finely tuned regulation in response to different environmental stresses. In addition, alternatively, the characterization of the impact of pesticides might also be addressed by using biological model, presenting an interest from the functional point of view, in order to develop new ecotoxicogenomic tools dedicated to monitor the efficiency of soil ecosystemic services.

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