Final Report Summary - LOVE-TO-HATE (Pesticides: Felicity or curse for the soil microbial community?)

The LOVE-TO-HATE project (http://lovetohate.bio.uth.gr) is an Industry – Academia Partnership and Pathways project which developed a research and training network of industrial (AEIFORIA srl., ENOVEO sarl) and academic partners (University of Thessaly, University of Patras, INRA). The network enabled the mobility of 8 early stage researchers, 8 experienced researchers and 4 most experienced researchers and the recruitment of 4 experienced researchers. The fellows studied the interactions of pesticides with soil microorganisms with main research aims (a) the development of a novel procedure for assessing the toxicity of pesticides on soil microorganisms, in light of recent opinions by EFSA stressing the need for revision of the relevant risk assessment scheme, (b) the investigation of the genetic mechanisms driving the accelerated biodegradation of pesticides in soil.

A tiered lab-to-field experimental approach was employed to assess the toxicity of 3 model pesticides (chlorpyrifos, isoproturon and tebuconazole) on soil microorganisms. Lab microcosm studies constituted a worst case exposure scenario (Tier I), whereas field tests represented a more realistic exposure scenario for soil microorganisms (Tier II). Lab tests involved the application of pesticides at four dose rates, x0, x1, x2 and x10 the recommended dose rate, whereas field testing involved application of x0, x1, x2 and x5 the recommended dose rate. The levels and the duration of exposure to the model pesticides but also to major metabolic products were determined analytically. Particular attention was given to the soil transformation of tebuconazole for which little was known. This was determined by combining suspect screening time-of-flight mass spectrometry with in silico molecular typology. Effects on the soil microbial abundance and function were determined using a range of standardized tools like determination of soil enzymatic activities (using fluorometric high-throughput tests), measurements of the abundance of key functional microbial groups involved in C (genes involved in the degradation of natural aromatic compounds), N (ammonia-oxidizers, denitrifiers) and S (sulfur-oxidizing bacteria) cycling and 11 major microbial taxa.

The impact of pesticides on the diversity of soil bacteria and fungi were determined using amplicon sequencing via Illumina Miseq and PhyloChip®. Overall, did not induce any clear dose-dependent effects with the exception of microbial groups involved in N cycling like ammonia-oxidizers which were significantly affected by chlorpyrifos and its main derivative TCP and denitrifiers which were affected by the metabolites of isoproturon. The three pesticides did not have any clear dose-dependent effect on the diversity of bacteria, whereas significant changes in the diversity of the fungal community were evident although no clear dose-dependent patterns were seen. Overall the three model pesticides tested, and their metabolites, did not induce consistent effects on the function and diversity of the soil microorganisms. The use of advanced and standardized molecular tools allows a comprehensive assessment of pesticide effects on the abundance and function of soil microorganisms, whereas effects on microbial diversity still needs standardization at both technical and data analysis level. The project tackled further open issues regarding the assessment of the soil microbial toxicity of pesticides. Follow up tests assessed the soil microbial toxicity of the model pesticides in 3 soils obtained from the three geographical zones of Europe (South, Central and North) in accordance with the EU pesticide regulatory framework. Although different dissipation patterns were observed in the different soils and at different dose rates, no effects on the abundance and functions of soil microorganisms were observed. The outcome of all these studies led us to propose a novel tiered assessment scheme for assessing the soil microbial toxicity of pesticides (see Figure 1 attached). This is based on two tiers involving pesticide testing against natural microbial assemblages in microcosm and field scale. An in vitro assessment of the toxicity of pesticides on representative and sensitive ammonia-
oxidizing microorganisms and arbuscular mycorrhizal fungi could be implemented as an initial conservative tier before testing against natural assemblages of soil microorganisms at lab and field scale (tiers I and II). However, certain aspects of in vitro testing need standardized before implementation in pesticide environmental risk assessment. The proposed risk assessment scheme is expected to have a strong impact on the revision of the relevant regulatory framework which is on the way at EU level and our studies be a benchmarking on the forthcoming and most needed revision of this regulatory framework.

The LOVE TO HATE project also investigated the complex mechanisms driving the adaption of a fraction of the soil microbial community to rapidly transform pesticides. Advanced 16S rRNA amplicon sequencing were used to identify soil bacteria which are benefited after a fresh addition of oxamyl (carbamate insecticide) and isoproturon (phenyurea herbicide) in soils exhibiting enhanced biodegradation of these compounds. DNA-based amplicon sequencing analysis did not distinguish microorganisms which responded to oxamyl and isoproturon additions. Whereas when RNA-based amplicon sequencing was performed it was evident, especially in the oxamyl-treated soils, that α-, γ-proteobacteria, Acidobacteria, Actinobacteria and Myxococcales from δ-proteobacteria were stimulated.

The LOVE-TO-HATE project developed a functional metagenomic pipeline for soils and other organic matrices exhibiting accelerated biodegradation capacities against pesticides. Through this pipeline novel pesticide hydrolytic enzymes will be isolated and used in downstream industrial applications in environmental clean-up, human health and agriculture. This pipeline was employed in a soil exhibiting enhanced biodegradation of carbamates and in an organic biomass from an on-farm biopurification systems. In total over 50000 fosmid clones were assayed for esterases and monoxygenases and 12 positive clones were sequenced. Novel esterases with putative role in the hydrolysis of pyrethroids and a known demethylating monoxygenase PdmAB involved in the metabolism of phenylureas were detected. The functional metagenomic pipeline developed is the first to be used directly for the isolation of novel pesticide biocatalysts from soils and is expected to be exploited further for the isolation of enzymes with biotechnological interest for future industrial exploitation.

The LOVE TO HATE project developed a prototype functional microarray (PestiChip) which could provide an estimate of the biodegradation genetic potential of the microbial community in agricultural soils. It covers a total of 21 genes involved in the degradation of five major pesticide groups like carbamates, organophosphates, triazine herbicides, phenylureas and phenoxy alkanoic acids. The prototype has gone through two validation tests with DNA obtained from bacterial strains and soils carrying the target genes and the finalized version has 9486 probes. Its commercialization will prevent unnecessary crop protection failures due to the development of enhanced biodegradation problems especially in crop monoculture regions where a limited number of pesticides are used throughout the years facilitating microbial adaptation, rapid biodegradation of the applied pesticides and efficacy loss.

Through the Love-to-Hate project a range of dissemination and outreach activities were undertaken: (a) the Summer School “Pesticides and Soil Microbes in the Era of Omics” which was attended by the fellows of the project but also from young researchers from EU and Iran, (b) the Love-to-Hate Open Day, (c) visits to schools by the fellows, (d) the production of training videos, podcasts and annual e-newsletters. The findings of the Love-to-Hate project are expected to have a major impact on the EU pesticide regulation enabling the comprehensive assessment of the toxicity of pesticides on soil microorganisms whose pivotal role in ecosystem functioning is well-documented.