TerbAzineBiorem – Studies on the efficacy and scale-up of bioremediation strategies for soils contaminated with the herbicide terbuthylazine

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s-Triazine herbicides (e.g. atrazine-ATZ, terbuthylazine-TBZ) have been used extensively worldwide for weed control in a variety of crops. In the European Union (EU), about 3600 tons of *s*-triazine herbicides have been used each year, representing 4.3% of the total of herbicides. ATZ has been recently banned from most of the EU countries, though it is still intensively used in the USA, Africa, Latin America, Asia and Australia. On the other hand, TBZ recently gained agricultural relevance, replacing ATZ in diverse regions of the world including the EU. Even though it has been considered as having more environment-friendly properties than ATZ (e.g. lower mobility and bioavailability in soil), its higher sorption to soil organic matter may increase the potential to reach waters and sediments due to runoff and drainage events, with special relevance for sites where careless disposal or accidental spills may occur. Importantly, concerns regarding the toxic impact of TBZ in aquatic and terrestrial ecosystems are gaining evidence. The wide use of ATZ and its potential to cause environmental hazard effects, have promoted research over degrading microorganisms and efficient bioremediation tools for polluted soils aiming to reduce ATZ contamination to safe levels. For example, one strategy for transforming ATZ into less harmful end-products in soil, whose efficacy was recently demonstrated by us, is by joining bioaugmentation (with the well-characterized ATZ-mineralizing bacteria *Pseudomonas* sp. ADP (*P.* ADP)) and biostimulation with additional carbon sources such as citrate or succinate. *P.* ADP is also able to mineralize TBZ, but with a slower rate than for ATZ. *Arthrobacter aurescens* TC1 (*A.a* TC1) is a powerful *s*-triazine degrading bacteria and with a wider range of substrates (TBZ included) than *P.* ADP. It was recently offered to us by Prof. Lawrence Wackett (Univ. Minnesota, USA). Contrary to ATZ, the transformation and fate of TBZ in environmental compartments and efficient remediation strategies have been investigated more sporadically. Therefore, the present project aims to improve the knowledge about the biodegradation of triazine herbicides in soils, with emphasis on the less well known TBZ. Its ultimate goals are to optimize and to scale-up bioremediation strategies that will help to achieve rapid detoxification of soils polluted with TBZ-based herbicidal formulations (due mainly to overuse, careless disposal or accidental spills) and thus prevent contamination of adjacent freshwater compartments with TBZ and its toxic chlorinated metabolites. The project will take profit from the scientific expertise gained by the present multidisciplinary research team that combines complementary skills; indeed, during the last years, this team has contributed for the development, optimization and ecotoxicological assessment of a bioremediation strategy to cleanup natural soils spiked with commercial formulations of ATZ. To accomplish project objectives, we intend to address the following issues: 1) to isolate from agricultural soils novel efficient triazine-degrading bacterial strains and to get clues on the involving genes and bacteria physiology (IST team); 2) to study the influence of

nutrient limitation (e.g. C, N, P sources) and of relevant environmental factors (e.g. pH, moisture, soil aging), on TBZ-biodegradation in soils by *A.a* TC1 and P. *ADP*, either alone or in consortium; and to extend these studies to novel relevant herbicide-degrading bacteria (IST team); 3) to establish and optimize bioremediation strategies for the cleanup of soils spiked with TBZ-based herbicidal formulations at doses mimicking situations arising from agricultural application up to worst-case scenarios, at microcosms scale (IST and IMAR teams); 4) to scale-up and examine at semi-field and field scales the bioremediation technologies developed (IMAR and IST teams). An innovator aspect of the project will be the ecotoxicological evaluation of the efficacy of the bioremediation processes to be developed, based on ecologically relevant toxicity bioassays performed on the soils treated with the bioremediation tools under study and on water samples (including soil eluates, leachates and/or runoffs), compared to samples from non-bioremediated ones (IMAR team). It is anticipated that the project will develop knowledge and technology that will contribute to improve the efficacy of biological strategies with potential for the reclamation of sites polluted with triazine herbicidal formulations and consequently the protection of aquatic compartments from triazines contamination, to improve ecotoxicological monitoring methodologies to assess the efficacy of the bioremediation treatments, and to enhance information on the ecotoxicity of TBZ (and metabolites), with relevance in the field of Environmental Sciences and Technology.