Scientific Papers. Series A. Agronomy, Vol. LXV, No. 1, 2022
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

METHODS USED FOR ECOTOXICITY ASSESSMENT OF POLYMERIC PACKAGING MATERIALS

Elisabeta Elena POPA, Mihaela GEICU-CRISTEA, Paul Alexandru POPESCU, Amalia Carmen MITELUȚ, Mihaela Cristina DRĂGHICI, Silvana Mihaela DĂNĂILĂ-GUIDEA, Mona Elena POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaela_geicu@yahoo.com

Abstract

In recent years, the growing demand and use of polymeric materials resulted in a great waste disposal problem at a global level. Furthermore, polymeric materials can cause serious damage to the environment due to the fact that they are not biodegradable and persist in the environment for hundreds of years. In order to minimize the environmental impact, researchers developed various formulations of polymeric materials that can be obtained from natural resources and present properties such as biodegradability and biocompatibility, as substitutes to the traditional polymers. In addition to biodegradation tests, in order to determine their effect on the environment, ecotoxicity tests have been used. The aim of this study is to present various methods for polymeric materials ecotoxicity determination.

***Key words**: aquatic ecotoxicity, soil/compost ecotoxicity, polymeric materials.*

INTRODUCTION

In the conditions of the modern economy, characterized by the expansion and modernization of markets, by the more pronounced orientation of producers towards consumers, the packaging industry is booming, thus many companies are investing in designing sustainable food packaging materials (Hermabessiere et al., 2017). Packaging manufacturers are focusing on technologies that reduce the consumption of raw materials, increase labour productivity in both packaging and product packaging, increase the shelf life and shelf life of the product, combine various types of materials to increase packaging performance, reduce environmental impact (Sforzini et al., 2016).

Traditionally, the vast majority of the food packaging materials on the market are made from petroleum-based polymers (Gutierrez & Alvarez, 2018), thus creating an environmental problem. The lack of compostability and biodegradability capabilities of petroleum-based packaging materials have made? possible the development of newly bio-based and eco-friendly packaging materials (Kuai et al., 2020).

Biodegradable biopolymers are a current field of scientific research of great ecological, scientific and economic importance. In the context of concerns related to the preservation of the environment, the recovery and recycling of plastic waste, the replacement of traditional synthetic polymers with polymers obtained from renewable and ecological resources and the development of biodegradable polymer blends, with applications in agriculture, medicine, food industry etc. are topics of great interest and research currently. High molecular weight macromolecules, containing covalent bonds, are not easily decomposed naturally under the conditions provided by waste management infrastructures (Mitrus et al., 2009). With a growing consumption market, sustainability becomes a very important factor which depends on the attitudes adopted by the production sector in order to achieve cleaner manufacturing processes. Consuming consciously is a practice which has grown nowadays at a global level and producers should adopt a proactive approach for pollution monitoring and the impact that their products have over the environment (Marzullo et al., 2018). Therefore, the sustainability of packaging materials from an environmental point of view should be evaluated with tools/ methods that allows the estimation of environmental impacts that could be produced by packaging during all its life stages (Feo et al., 2022).

Ecotoxicity tests are nowadays required for the risk-assessment of existing and newly developed chemical-based products and pesticides in order to test their impact on the environment. Usually, the standards of which the ecotoxicity tests are made differ slightly from one country to another. There is a two-way classification of ecotoxicity testing methods, the first one being lower-tier testing in laboratory scale after standardised conditions, which consists of single cohorts of test species from different environmental categories. The second category is known as higher-tier testing which is more intricate, thus the ecotoxicity test are made on a larger scale population or more species (Ratte et al., 2003). In order for the ecotoxicity testing to be justiciable, attributes like reproducibility and repeatability are a must, thus all testing is performed under accepted standards, guidelines and Good Laboratory Practice (OCED, 1998b). Another important part of the validation of ecotoxicity testing is the statistical analysis of the obtained results (Sparks, 2000). When selecting the appropriate statistical analysis method a few key factors must be taken into consideration: measurement variables, ranked variables, attributes and derived variables (Ratte et al., 2003).

MATERIALS AND METHODS

To determine the impact that polymeric materials have over the environment, ecotoxicity tests can be performed under laboratory conditions. Choosing the method and test species depends on the investigated specific ecosystem. For example, for terrestrial environments could be used as test species various soil organisms like microorganisms or terrestrial plants and for aquatic ecosystems algae, crustaceans or fish could be used as test organisms (Haider et al., 2018).

RESULTS AND DISCUSSIONS

Soil/compost ecotoxicity

There is an increased interest in the effects over the environment of composting and soil degradation at a global level. This is why all the testing protocols that have been developed for the characterization of biodegradable plastics and packaging include the assessment of ecotoxicity potentially originated after biodegradation or composting (Sforzini et al., 2016; Vaverková et al., 2018). From an environmentally protection point of view, compost should not contain substances that could be harmful in any way. Therefore, several methods can be used in order to determine soil/compost ecotoxicity, by exposure of different organisms to the tested material. Some research are based on studies regarding bioaccumulation - results from direct exposure (the accumulation of a contaminant in a tested organism), bioconcentration - results from direct contact like respiration or skin contact (the accumulation of a contaminant in an specific organism only from its environment in which it lives) or biomagnification (the accumulation of the contaminant in one organism is higher than the concentration in an organism that is on a lower trophic level) (Bour et al., 2015).

Mitelut & Popa (2011) evaluated the ecotoxicity of compost obtained after composting of six types of biodegradable materials for 90 days, using seed germination bioassay. Compost extracts of 25%, 50%, 75% and 100% were obtained as the test solution for *Raphanus sativus* seeds, and their germination capacity and root growth were monitored. No direct correlations with the composition of the tested materials could be found; however, it was observed a direct correlation between the phytotoxicity and the concentration of the compost extract, all tested extract being more toxic than the control. In another study, Mitelut et al. (2019) determined the ecotoxicity of the soil resulted after the biodegradation process of some polymeric packaging materials using seed germination bioassay on radish (*Raphanus sativus*) and cucumber (*Cucumis sativus*) seeds. The results of the study showed that the tested soils had no toxic effect over the tested seeds development, obtaining a germination index over 80%, being higher for the samples compared to the control soil.

Gutiérrez et al., 2012, studied the biodegradability and ecotoxicity proprieties of some newly developed packaging materials derived from thermoplastic gluten (TPG) developed under reactive extrusion and thermomolding. The films consisted of TPG, TPG/poly (ε-caprolactone) (PCL) and TPG/PCL + chrome octanoate (Cat) as food grade catalyst. Thermal, physicochemical, thermal, mechanical and environmental tests were made on all the developed films. The ecotoxicity method used in the analysis was performed by comparing the growth of primary roots of lettuce treated with the powder of developed film system (0.1 and 1 mg/mL) compared to samples that grew in water. The results showed that all samples were biodegradable under vegetal compost storage after a period of 90 days. None of the analysed materials showed any ecotoxicity during testing.

Martin-Closas et al., 2014, carried out a study in which an in vitro system for ecotoxicity analysis of different plastic constituents was developed. Seeds of tomato, *Lycopersicon esculentum*, and lettuce, *Lactuca sativa* were subjected to the plastic particles during mulch biodegradation, and different parameters (seed germination period, changes in culture media and plant growth) were monitored over time. The results of the in vitro bioassay showed that the under exposure to 50 and 500 mg l^{-1} of adipic acid in the culture medium, tomato growth was severely restricted. Opposite to this, the samples exposed to butanediol and lactic acid presented a better growth rhythm that the control samples. It was shown that the roots are more prone to be affected by exposure chemicals than the shoots and leaves.

The ecotoxicity of four iron-based nanomaterials, loaded with iron oxide and nanoscale zero valent iron (nZVI), provided directly by the manufacturer was assessed by Hjorth et al. (2017). Eight ecotoxicity tests were performed over radish (*R. sativus*), ryegrass (*L. multiflorum*), algae (*P. subcapitata*, *Chlamydomonas* sp.), bacteria (*V. fisheri*, *E. coli*), earthworms (*E. fetida*, *L. variegatus*) and crustaceans (*D. magna*). The results showed that the materials did not show any ecotoxicity at concentrations up to 100 mg/L. Only one Fe nanomaterial, namely FerMEG12 particles, presented toxicity effects to aquatic organisms, thus being classified as potentially toxic.

In a study performed by Jiang et al. (2019) higher plant *Vicia faba* root tips were exposed to different amounts of polystyrene fluorescent microplastics. The results of the study indicate that polystyrene microplastics can be toxic to *V. faba*, especially at the highest concentration used (100 mg/L).

Abe et al., 2022, carried out a research study in which newly developed bioplastics from xylan/ starch combining α-cellulose and holocellulose were developed and tested for ecotoxicity and biodegradation proprieties. The disintegration of the bioplastic was assessed in soil and compost, thus the method consisted in burying the bioplastic samples (cut into 3.5 x 3.5 cm squares) and quantifying the resulting mass loss after 13 days of incubation. The ecotoxicity study was made with the soil resulted from the biodegradation process and cucumber seeds. One gram of each soil sample was washed with 5 mL of deionized water and the filtered solution was transferred on filtered paper disks in a Petri dish. Eight cucumber seeds were placed on the filtered paper and the percentage of germination and inhibition was calculated. The results showed that the tested bioplastics presented no ecotoxicity over the growth of the cucumber seeds.

The ecotoxicity of compost containing biodegradable polymeric materials were determined by Kopec et al. (2013) using five test organisms. The study shows germination and root growth inhibition, *Heterocypris incongruens* growth inhibition *Vibrio fischeri* luminescence inhibition in composts containing biopolymers.

Due to limited information regarding soil ecotoxicity of microfibres, Kwak & An (2022) compared the effect of short lyocell microfibers, short polypropylene microfibers, and long polypropylene microfibers on the earthworm *Eisenia andrei*. After 21 days of exposure, the study showed that the earthworm was negatively affected at high exposure concentration (1 g/kg dry soil). All tested microfibres reduced earthworm survival, more toxic being the short polypropylene microfibres.

Aquatic ecotoxicity

Aquatic ecotoxicity is performed in to obtain information on toxicity for in order to determine the risks to both aquatic and terrestrial environments unless the information regarding physical and chemical properties of a specific substance suggest that it might divide onto aquatic sediments or soils. Aquatic biological indicators that are usually used in these tests are represented by algae or fish (Crane et al., 2008).

A study on the ecotoxicity of micro-debris derived from the aging process of polyvinylchloride and Mater-Bi® plastic materials on freshwater organisms was carried out by Magni et al., 2020. The sub-lethal effect of the above plastic micro-debris was assessed on *Dreissena polymorpha*, which can be found in freshwater systems. 1 mg/L of polyvinylchloride (PVC) and Mater-Bi® powders were introduced in semi-static conditions to *D. polymorpha* and a suite of biomarkers analysis (oxidative damage, genotoxicity and cellular stress) were performed over a period between 6 and 14 days. The results showed that there were no relevant sub-lethal effects after exposure to micro-debris plastics.

Vijayakumar et al., 2016 studied the ecotoxicity of samples made from sodium alginate stabilized silver nanoparticles using *Ceriodaphnia cornuta*, which is a crustacean found in fresh waters. For the ecotoxicity testing, *C. cornuta* was cultured in synthetic freshwater at temperatures around 23°C, pH of 7.9 and with a density of maximum of 50 animals per litre. The toxic effect of the sodium alginate stabilized silver nanoparticles was performed by exposing the *C. cornuta* to different concentrations of the tested nanoparticles. To highlight the potential toxic effect, the number of dead organisms after 24h of exposure to the nanoparticles. The results showed that lethal effect of the nanoparticles was at 40 $\mu g\ L^{-1}$.

The cytotoxicity of nanomaterials from zinc oxide on murine macrophages was studied by Vijayakumar & Vaseeharan (2018). Three different quantities of (20, 50 and 75 µg/ml) of the analysed nanomaterials were subjected to RAW264.7 murine macrophages cell lines and the cytotoxic effect was studied under phase contact microscopy over the apoptotic cell morphology. The control samples consisted of collagen and bare zinc acetate. The results were gathered after 48 hours of exposure and showed that both the control samples and the test samples did not exhibit any cytotoxic effect on the morphology and cell viability of the murine macrophage cells.

Graphene (like graphene oxide) based materials can be used in various applications which can lead to potential release and occurrence into aquatic environments. Therefore, ecotoxicity tests were performed by Evariste et al. (2020) using a consortium of algae and bacteria as primary producers, then as primary consumers and decomposers - chironomid larvae and as secondary consumers larvae of the amphibian *Pleurodeles waltii* were used. In this study, changes in bacterial communities were observed, while in chironomids no toxic effects were determined.

Tiede et al. (2009) studied the environmental effect and ecotoxicity of nanoparticles through analytical techniques such as dynamic light scattering, microscopy and size separation technologies. The test conditions must be selected carefully as the medium will greatly influence the ecotoxicity of the nanoparticles. When conducting the ecotoxicity experiments several parameters must be monitored, such as characteristics and concentrations of the nanoparticles as well as the conditions of the environment. The main factors that can greatly influence the results of the aggregation, stabilization and ecotoxicity of nanoparticles are: type of aquatic system, the presence of humic substances, pH alterations, light conditions, sediments, type of test vessels. The conclusions of the study revealed that it is not entirely known in the present what impact do the nanoparticles have on the environment and further testing must be done.

Capolupo et al. (2020) determined the toxicity of plastic/rubber leachates on *Raphidocelis subcapitata* (freshwater), *Skeletonema costatum* (marine) and the Mediterranean mussel *Mytilus galloprovincialis*. The results showed that with increasing, the leachates ranged from slightly to highly toxic to algae and mussels, therefore additional tests are necessary for a better comprehension of the total impact of chemical associated with plastic on aquatic ecosystems.

The effects of glyphosate-based herbicides, glufosinate ammonium-based herbicides and polyethylene microplastic particles on *Scinax squalirostris* tadpoles were determined by Lajmanovich et al. (2022). The study concluded that the increased ecotoxicity and the changes in the biochemical parameters of *S. squalirostris* tadpoles exposed to both herbicides mixed with the microplastics and their occurrence in water bodies represents in fact an ecotoxicological risk for amphibian tadpoles.

Choi et al. (2014) determined the ecotoxicity of water-solubilized aminoclay nanoparticles on the bioluminescent marine bacteria *Vibrio fisheri*, the crustacean *Daphnia magna* and on eukaryotic microalga *Pseudokirchneriella subcapitata*. The results showed that aminoclay nanoparticles could be used as algae inhibition with concentrations lower than 100 mg/L with mild affection or no effect on other organisms' inclusive zooplanktons.

Villa et al. (2020) aimed to perform a study in order to determine the ecotoxicological effect of CeO_2NPs with different surface modifications characteristic to nanoparticles bio-interactions with naturally occurring molecules in the water environment. Further, ad hoc synthesis of CeO_2NPs with different coating agents (alginate and chitosan) was performed. The ecotoxicity was assessed using *Aliivibrio fischeri* and *Daphnia magna*. The different coatings significantly influenced the toxic effects of CeO_2NPs. For example, the alginate coated CeO_2NPs activated oxidative stress in *D. magna*, while the ones coated with chitosan induced hyperactivity. Our findings emphasize the role of environmental modification in determining the NP effects on aquatic organisms.

Four (one in liquid phase and three in solid phase) water soluble polymers based on polyacrylic acid were tested in terms of ecotoxicity by Rozman & Kalčíková (2021) using aquatic plant *Lemna minor*, crustacean *Daphnia magna*, microalga *Pseudokirchneriella subcapitata*, bacterium *Allivibrio fischeri*, and a mixed bacterial culture of activated sludge. The effect on the tested organisms was low or moderate, the liquid water soluble polymer having a specific toxic effect on the bioluminescence of *Allivibrio fischeri* and on the oxygen consumption of the nitrifying microorganisms (inhibition) from the activated sludge.

CONCLUSIONS

This paper aimed to present the ecotoxicity methods used for the determination of environmental impact of polymeric packaging. Two major directions were identified within this study, namely ecotoxicity performed in aquatic environments (marine, fresh water) and ecotoxicity of soil/compost resulted after biodegradation/composting of polymeric materials. These tests are performed based on the necessity and further use of the teste materials. However, scientific data in this area is scarce, more research is needed in order to clearly establish methods for ecotoxicity determination.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI - UEFISCDI, project number 379PED/2020, acronym BIOMATFOOD, within PNCDI III.

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