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DOCTORAL THESIS

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ABSTRACT

Plastics is an inevitable part of our daily life, hence its usage will continue to grow. Multiple and prolonged use and abrasion of the plastic surface might result in partial deterioration of plastic to micro-sized particles – microplastic (MP). Recently, it has been observed that MP's multi-faceted structural and surface properties makes it an adsorbent for numerous aquatic pollutants such as pharmaceuticals and pesticides, defined under the EU water protection legislation as contaminants of emerging concern (CECs), are estabished. Their hydrophobicity, polarity and low-biodegradability makes them readily adsorbable at polar, hydrophobic, aged and degraded specific surfaces of MP, contributing to the overall toxicity of such binary systems. Besides, in environmental conditions MP might leach additives and constituents that are often found toxic and hazard.

The aim of this dissertation was to investigate the influence of thermal- and photo-aging of polyethylene terephthalate (PET) and polypropylene (PP) on the changes in morphology and structure of polymer materials and the overall ecotoxicity of PET and PP MPs in water. The characterization of pristine and aged MPs was performed by Fourier-transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), as well as Water Contact Angle (goniometer) and the Branuer Emmett Teller (B.E.T.) specific surface area measurements. In order to investigate the fragmentation of plastic materials into MPs, grinding was performed in a single-drum cryogenic mill, while size distribution of obtained MPs particles was determined by the sieve shaker with five sieves of different mesh sizes to separate the particles according to their size. Adsorption of selected CECs, pharmaceutical diclofenac (DCF) and pesticide atrazine (ATZ), was investigated on both pristine and aged MPs according to the computational/empirical approach employing full factorial design (FFD) as the experimental plan in the combination with response surface modelling (RSM). Furthermore, pristine and aged MPs along with PP waste films from commercial food and cosmetic packaging, were submitted to leaching tests in order to study possible migration of additives and consituents. The evaluation of the aquatic toxicity of the samples after the adsorption and leaching tests was tested on the marine bacteria *Vibrio fischeri* (*VF*), freshwater crustacean *Daphnia magna* (*DM*) and microalgae *Raphidocelis subcapitata* (*RS*), following the procedures disclosed in ISO 11348-3:2007, ISO 6341:2012 and ISO 8692:2012 standards, respectively. Finally, the leachates were tested for their biodegradability, along with glucose as readiliy biodegradable substrate.

Direct correlation can be drawn between prolonged aging and increase in toxicity of MP. Characterization revealed severe degradation of material; thermal-aging affected the structure, while photo-aging caused the surface alterations of PET and PP. Hence, changes in surface morphology such as cracks, fractures, notches and bumps were observed. The increase in crystallinity of PET and PP resulted in changes of brittleness and accelerated fragmentation to smaller MP's particles. It was found that the hydrophobicity increased, while specific surface decreased; the exception was photo-aged PP. Adsorption of CECs onto MPs is correlated with the changes in the surface morphology enhanced with the photo-aging. Adsorption of CECs was driven by hydrophobicity and specific surface, as well as smaller MP particle sizes as a result of accelerated and prolonged aging period. RSM approach revealed that temperature is more significant parameter than MP dosage for CECs adsorption. It can be concluded that aquatic toxicity generally increases with the prolonged exposure to aging, especially valid for the photo-aged MPs. However, some exceptions were observed depending on the type of MPs, CECs and targeted toxicity test organism. Individual CECs showed high toxicity resposnes toward *DM*, while slightly lower toward *RS* were recorded. It was also found that *DM* as shown to be the most sensitive to exposure to MPs with and without CECs. Aging of MPs resulted with the accelerated leaching of constituents and organic additives for both PET and PP. Hence, (heavy) metals originated from polymerization process and organic additives were identified in leachates, resulting with toxic responses in cases of all three tested microorganisms. However, *DM* was shown to be the most sensitive to leachates originated from PP MPs, while in the case of PET leacheates other two test microorganisms (*VF* and *RS*) were more sensitive in toxicity response than DM. The biodegradability of glucose was inhibited by the presence of metals and organics in leachates originated from both thermal- and photo-aged PET and PP MPs.

Key words: Ecotoxicity, Microplastic, Priority Pollutants, Adsorption, Leaching

SAŽETAK

Plastika je neizbježan dio svakodnevnog života i smatra se kako će njezina uporaba nastaviti rasti. Višestruka i dugotrajna uporaba te abrazija površine plastike može rezultirati djelomičnom fragmentacijom na čestice mikro veličine – mikroplastiku (MP). Nedavno je uočeno kako mikroplastika ima brojna strukturna i površinska svojstva koja je čine adsorbentom za brojna onečišćivala pristutna u vodi kao što su farmaceutici i pesticidi, koji su prema zakonodavstvu vodnom zakonodavstvu EU-a definirani kao onečišćivala koja izazivaju zabrinutost (eng. *Contaminants of Emerging Concern*, CEC). Njihova hidrofobnost, polarnost i slaba biorazgradivost čine ih toksičnima, a lako se apsorbiraju na polarnu, hidrofobnu, ostarenu i degradiranu specifičnu površinu MP. U okolišnim Pjetima može doći i do izluživanja (eng. *leaching*) aditiva te konstituenata koji mogu biti toksični i opasni za organizme.

Cilj ove disertacije bio je istražiti utjecaj termo- i foto- starenja polietilen tereftalata (PET) i polipropilena (PP) na promjenu morfologije i strukture materijala te ukupnu promjenu ekotoksičnosti mikroplastike u vodi. Karakterizacija nestarene i starene MP provedena je pomoću infracrvene spektroskopije s Fourierovom transformacijom (FTIR), diferencijalne pretražne kalorimetrije (DSC), termogravimetrijske analize (TGA), skenirajuće elektronske mikroskopije (SEM), kao i goniometra za određivanje kontaktnog kuta vode te mjerenjem specifične površine na temelju Branuer Emmett Teller (B.E.T.) modela. Kako bi se istražila fragmentacija plastike u MP, mljevenje je provedeno u kugličnom kriogenom mlinu, dok je raspodjela veličine dobivenih MP čestica određena pomoću vibracijske tresilice s pet sita različitih veličina otvora za izdvajanje čestica prema njihovoj veličini. Adsorpcija CEC-a (DCF i ATZ) ispitana je i na nestarenoj i starenoj MP u skladu s računalnim/empirijskim pristupom na temelju punog faktorskog dizajna (eng. *Full Factorial Design*, FFD) u kombinaciji s metodologijom odzivne površine (eng. *Response Surface* Methdology, RSM). Nadalje, nestarena i starena MP zajedno s obojenim komercijalnim PP-om podvrgnuti su testovima izluživanja kako bi se ispitala moguća migracija metala te BPA. Glavni fokus ove disertacije bila je procjena toksičnosti uzoraka u vodi nakon provedenih testova adsorpcije i izluživanja i to na morskim bakterijama *Vibrio fischeri* (*VF*), slatkovodnim račićima *Daphnia magna* (*DM*) i mikroalgama *Raphidocelis subcapitata* (*RS*), prema standardima ISO 11348-3: 2007, norme ISO 6341:2012 i ISO 8692:2012. Uz to ispitana je i biorazgradivost uzoraka nakon testa izluživanja.

Može se odrediti izravna veza između starenja te povećanja toksičnosti MP. Karakterizacija je ukazala na degradaciju materijala, gdje je termooksidacija utjecala na strukturu, dok fotooksidacija uglavnom na površinske promjene PET-a i PP-a, što je izraženo

u vidu promjene površinske morfologije s vidljivim pukotinama, lomovima, zarezima i neravninama. Također, povećanje kristalnosti PET-a i PP-a rezultiralo je promjenom lomljivosti te ubrzanoj fragmentaciji na manje čestice MP. Može se reći kako se hidrofobnost povećala, dok se specifična površina smanjila, osim u slučaju starenog PP-a. Adsorpcija CEC-ova na površinu MP u korelaciji je s promjenama morfologije površine nastale uslijed fotooksidativnog starenja. Adsorpcija hidrofobnih CEC-ova potaknuta je hidrofobnošću i specifičnom površinom, kao i malim veličinama čestica starene MP. RSM ukazuje na to kako je za adsorpciju temperatura (niža) značajniji parametar od udjela MP. Može se reći kako se općenito može odrediti veza između povećanja toksičnosti i starenja, osobito fotooksidacijskog tipa. Općenito starena MP uzrokuje veći toksični odgovor od nestarene, dok adsorbirani CEC izaziva antagonističke učinke, smanjujući zajedničku toksičnost u usporedbi s pojedinačnom. Međutim, primijećene su neke iznimke ovisno o vrsti MP-a, CEC-ova i ciljanog organizma za ispitivanje toksičnosti. Može se reći kako je u većini slučajeva uočeno kako $CaCl_2$ ima stimulativni učinak smanjujući toksičnost kada je prisutan u smjesi. Također, individualni CEC pokazuje visoku toksičnost prema *DM*, a nešto nižu prema *RS*. Također je utvrđeno kako je u slulaju MP *DM* najosjetljiviji organizam. Starenje plastike rezultiralo je pojačanim izluživanjem konstituenata PET-a i PP-a. Biorazgradivost glukoze je u oba slučaja smanjena. Toksičnost je drugačija u slučaju sva tri materijala, dok je *DM* najosjetljiviji organizam u slučaju izluženih uzoraka PP-a, dok je *VF* zajedno s *RS* u slučaju izluženih uzoraka PET-a.

Ključne riječi: ekotoksičnost, mikroplastika, prioritetna onečišćivala, adsorpcija, izluživanje