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CONTAMINATION OF FRESHWATER ECOSYSTEMS OF MONTENEGRO WITH MICROPLASTICS: FIRST OBSERVATIONS ON OCCURRENCE, ABUNDANCE, SPATIAL PATTERNS, IDENTIFICATION AND ECOLOGICAL ASSESSMENT

Doctoral dissertation

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KONTAMINACIJA SLATKOVODNIH EKOSISTEMA CRNE GORE MIKROPLASTIKOM: PRVA ZAPAŽANJA O POJAVI, BROJNOSTI, PROSTORNIM OBRASCIMA, IDENTIFIKACIJI I EKOLOŠKOJ PROCJENI

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Neda Bošković, PhD

Contamination of freshwater ecosystems of Montenegro with microplastics: First observations on occurrence, abundance, spatial patterns, identification and ecological assessment

ABSTRACT

In doctoral dissertation are presented the results of the content of microplastics in inshore sediments of the Zeta, Morača and Bojana rivers and Skadar lake, which belong to the Adriatic Sea basin. Sampling of surface coastal sediment of rivers and lake was carried out in two periodic cycles, autumn (2022) and spring (2023). Different anthropogenic activities, geographical location, terrain specificities and and opportunities to access them were the key criteria on the basis of which the locations for sampling on the examined water bodies were chosen. Were selected 4 to 6 locations per the river/lake where surface inshore sediments sediment sampling was performed. The selected locations for sampling the coastal sediment of rivers can be divided into three groups: the source, the middle of the river, and the mouth of the river into lake, river and sea, while the locations chosen for sampling the coastal surface sediment of the lake covered all sides of Skadar lake, with different degrees of anthropogenic influence, which belong to the territory of Montenegro.

The objectives of the research were based on: (1) determining the contamination of freshwater ecosystems, based on the microplastics presents in rivers and lake inshore sediments; (2) identification of microplastics, spatial distribution, microplastics fate and sources in river and lake sediments; (3) estimating the linear dependence of the influence of parameters such as: area, location and sampling season on the microplastics abundance; (4) assessment of the examined water bodies of the Adriatic basin as a source of microplastics on the Montenegrin coast and (5) identification of potential impacts of microplastics on aquatic organisms, the environment and human health.

Principal coordinate analysis (PCO), cluster analysis (CA) and permutation multivariate analysis (PERMANOVA) were statistical methods used to process the results. PCO and CA analysis were used to characterize the samples, i.e. the grouping of samples was identified in relation to the area, locations and sampling season, while on the basis of PERMANOVA the partitioning of variable factors was carried out as well as the assessment of the influence of the main factors.

In order to assess the environmental risk based on the presence of microplastics, the microplastic pollution load index (PLI) and the polymer hazard index (PHI) were applied.

Microplastics were identified in all examined sediment samples, where the presence of microplastics in the sediments of the investigated water bodies of the Adriatic basin was in the following order: Bojana (180 ± 53.5 MPs/kg dry sediment) > Morača (169 ± 113 MPs/kg dry sediment) > Skadar lake (153.4 ± 42.7 MPs/kg dry sediment) > Zeta (145 ± 110 MPs/kg of dry sediment). Hydrodynamic and ecological conditions, population density, tourist and fishing activities, wastewater discharge, inadequate solid waste management, as well as transboundary pollution are identified factors that can be linked to the sources, representation and distribution of microplastic in inshore sediments of rivers and lake of the Adriatic basin.

In the investigated sediment samples, the most abundant microplastic shape types were fibers and fragments, while granules were not identified in the sediments of Skadar lake. Medium-sized microplastics were the most abundant in the examined sediment samples, while blue, clear and red were the most abundant color categories in the examined sediment samples. Polypropylene was the most abundant type of polymer in Zeta and Bojana river sediments, while polyethylene was the most abundant type of polymer in the sediments of the Morača river and Skadar lake.

The results indicate that the rivers Zeta, Morača and Bojana, as well as Skadar lake, are one of the important sources of microplastics in the Adriatic Sea and represent secondary sources and reservoirs of previously accumulated microplastics. According to the PLI criterion, the overall ecological risk of microplastic in the sediments of the examined rivers and lakes was assessed as minor (category I), while according to the PHI criteria, the sediments of the examined rivers and lakes show a serious pollution trend (category V).

The aforementioned research was conducted for the first time in the freshwater ecosystems of Montenegro. The above results indicate that the investigated freshwater ecosystems are exposed to various anthropogenic activities, and the results of this research have a great potential to influence the behavior of the population and local communities in order to reduce the use of single-use plastic products, improve waste and wastewater management, as well as in the creation of potential rehabilitation measures.

Keywords: microplastics, Adriatic basin, sediment, rivers, lake, Montenegro
Scientific field: sustainable development, environmental protection
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Kontaminacija slatkovodnih ekosistema Crne Gore mikroplastikom: Prva zapažanja o pojavi, brojnosti, prostornim obrascima, identifikaciji i ekološkoj procjeni

PROŠIRENI REZIME NA SLUŽBENOM JEZIKU

Naziv disertacije "*Kontaminacija mikroplastikom slatkovodnih ekosistema Crne Gore: Prva zapažanja o pojavi, prostornim obrascima, identifikaciji, brojnosti, distribuciji i ekološkoj procjeni*" upućuje na ekološko stanje i kvalitet odabranih slatkovodnih ekosistema (rijeka i jezera) Crne Gore u pogledu sadržaja mikroplastike u površinskim obalnim sedimentima kao važnih ekosistemskih cjelina.

Pregled istraživanja

Plastika je promjenila ljudski život jer se koristi u različitim svrhama zbog svojih izvanrednih karakteristika (Bellasi et al., 2020). Međutim, usled povećane proizvodnje plastičnih materijala, njihovog dugog životnog vijeka, kratkog vijeka korišćenja plastičnih proizvoda dolazi do povećane količine plastičnog otpada koji stvara brojne izazove, kao i mogućnosti upravljanja ovom vrstom otpada (Bellasi et al., 2020). Zagađenje plastikom se distribuira od pola do pola, izazivajući veliku zabrinutost društvene i naučne zajednice.

Plastika je napravljena od sintetičkih organskih polimera, koji se obično proizvode polimerizacijom monomera dobijenih iz sirove nafte, prirodnog gasa ili uglja, odnosno iz izvora i rezervi fosilnih goriva (Ivleva et al., 2017). Osnovu plastike čine organske polimerne matrice, odnosno monomerne jedinice koje se ponavljaju (Andrady i Neal, 2009). Polimeri su hemijska jedinjenja čiji su molekuli veoma veliki, nalik dugim lancima, formirani od mnogo jedinica (monomera) spojenih kovalentnim vezama, gradeći polimere. Struktura i dužina polimernih lanaca diktira svojstva stvorene plastike. Aditivi su hemijska jedinjenja koja se dodaju u procesu proizvodnje plastike u cilju poboljšanja performansi, funkcionalnosti i svojstava starenja polimera. Svaki od aditiva ima posebnu ulogu u poboljšanju konačnih funkcionalnih svojstava plastičnog proizvoda (Hansen et al., 2013). Aditivi često imaju poznate negativne efekte po zdravlje ljudi i životnu sredinu (Bellasi et al., 2020). Rastući naučni dokazi i zabrinutost javnosti u pogledu toksičnosti korišćenih aditiva prilikom proizvodnje plastike doveli su do iznalaženja regulatornih mjera sa fokusom na ograničenje korišćenja opasnih i štetnih aditiva u plastici (Plastics Europe, 2021).

Mikroplastika (MP) predstavlja heterogenu grupu čestica, veličine od 0.1–5.0 mm, koja se klasifikuje po veličini, obliku, boji, hemijskom sastavu, gustini i izvoru (Sighicelli et al., 2018). Na osnovu izvora, MP se može podijeliti na primarnu i sekundarnu MP. Primarna MP je proizvedena MP u opsegu veličine od 0.1 – 5.0 mm, dok sekundarna MP nastaje usitnjavanjem (fragmentacijom) veće plastike na čestice reda veličine od 0.1 – 5.0 mm. MP predstavlja prijetnju biodiverzitetu zbog lakog unosa od strane akvatičnih organizama, a negativni efekti po žive organizme mogu biti fizički i hemijski. Fizički efekti se odnose na veličinu i oblik MP, a hemijski na hemijski sastav MP. Glavni putevi unošenja MP u ljudski organizam su ingestija (preko hrane i vode) i inhalacija (disajnim putevima) (Waring et al., 2018). MP ulaskom u ljudski organizam može izazvati niz neželjenih efekata i predstavljati potencijalni rizik po ljudsko zdravlje (Hwang et al., 2019). Međutim, potencijalni ekološki rizici kao i rizici po ljudsko zdravlje od strane MP su relativno nove oblasti istraživanja kojima se treba posvetiti posebna pažnja.

Prisustvo i postojanost MP je prepoznat problem koji se javlja u akvatičnim ekosistemima poslednjih 15 godina (Andradi, 2011). Većina studija izvještava o prisustvu MP u morskim ekosistemima, dok su saznanja o prisustvu i uticaju MP u slatkovodnim ekosistemima ograničena (Moore et al., 2011; Horton et al., 2017; Fahrenfeld et al., 2019). Danas je u porastu broj istraživanja koja se bave identifikacijom MP unutar slatkovodnih ekosistema, odnosno izvorima, putevima dospijevanja do slatkovodnih ekosistema i podzemnih voda, kao i potencijalnih uticaja na slatkovodene ekosisteme i zdravlje ljudi (Eriksen et al., 2013; Scherer et al., 2017; Turner et al., 2019; Lončarski, 2020).

Zagađenje slatkovodnih ekosistemima MP je veoma složeno jer slatkovodni ekosistemi obuhvataju jarke, potoke, rijeke, ušća, privremene i trajne močvare, bare, brane i jezera, od kojih svaki od njih ima različite karakteristike u smislu hidrologije, hemije, flore i faune, kao i slivove i obrasce korišćenja zemljišta. Štaviše, slatkovodni ekosistemi mogu da djeluju i kao prijemnik, ponor i transporter plastičnog zagađenja (Eerkes–Medrano et al., 2015; Horton i Dikon, 2018; van Emmerik i Schvarz, 2020).

Izvori MP u slatkovodnim ekosistemima su brojni (Simon–Sánchez et al, 2019), a najznačajnijim se smatraju: urbane sredine (otpadne vode iz domaćinstava, oticanja sa gradskih i drumskih površina, deponije i divlja smetlišta, neadekvatno upravljanje plastičnim otpadom), različite industrije i industrije proizvodnje plastike, atmosferska prašina, ribolov, poljoprivrede aktivnosti i dr. (Matjašič et al., 2022).

Studije ukazuju da slatkovodni ekosistemi igraju važnu ulogu u transportu MP. Slatka voda se smatra jednim od glavnih izvora MP u mora i okeane i glavnim transportnim vektorom plastičnog otpada sa kopnenih izvora (Iannilli et al., 2020), pa je proučavanje slatkovodnih ekosistema od velikog značaja za identifikaciju izvora zagađenja, dinamike, disperzije, akumulacija i sudbine MP (Dusaucy et al., 2021). Prisustvo MP u slatkovodnim ekosistemima ugrožava životnu sredinu, s obzirom da MP mogu ingestirati akvatični organizmi narušavajući njihovo zdravlje, uticajući na akvatični biodiverzitet i na čovjeka kao poslednje karike u lancu ishrane (Horton et al., 2017).

Plastika je veoma postojana, pa se procjenjuje da će trebati stotine godina da se degradira, a da će se najveća količina MP akumulirati u sedimentima (Klein et al., 2015). Zbog odustva ili spore biorazgradljivosti, toksičnosti i štetnosti, dugog životnog vijeka u životnoj sredini, ulasku u lanac ishrane, MP se smatra jednim od najozbiljnijih zagađivača akvatičnih ekosistema (Wang et al., 2016). Istraživanja ukazuju da su sedimenti veoma kontaminirani česticama MP (Hidalgo–Ruz et al., 2012; Vianello et al., 2013) i smatraju se „konačnim taložnikom" MP u akvatičnim ekosistemima (Nizzetto et al., 2016).

Koncentracija MP u sedimentima je znatno veće od koncentracije MP u vodenom stubu, zbog čega se smatra da su sedimenti veoma dobri indikatori za praćenje istorijskog i trenutnog zagađenja MP (Peng et al., 2018; Adomat i Grischek, 2020). Sedimenti mogu odražavati dugoročnu interakciju između slojeva voda–sediment obezbjeđujući važne informacije o dugotrajnoj akumulaciji, migraciji i sudbini zagađivača u akvatičnim ekosistemima (Andradi, 2011; Wang et al, 2017; Peng et al., 2018).

Do nedavno u Crnoj Gori ispitivanje prisustva MP u životnoj sredini nije bio predmet interesovanja i proučavanja, te nijesu postojali podaci i saznanja o istom. Danas postoje značajni naučni doprinosi o ispitivanju prisustva MP u morskom ekosistemu crnogorskog primorija (Bošković et al, 2022b, 2022c, 2023), ali do ove studije, ne postoje istraživanja o prisustvu MP u slatkovodnim ekosistemima Crne Gore.

Ciljevi disertacije

Ciljevi istraživanja zasnivali su se na: (1) ekološkoj procjeni slatkovodnih ekosistema, na osnovu sadržaja MP u površinskim obalnim sedimentima rijeka i jezera; (2) identifikaciji MP, prostorne distribucije, izvora i sudbine MP u rječnim i jezerskim sedimentima; (3) procjeni linearne zavisnosti uticaja parametara poput: područja, lokacije i sezone uzorkovanja na koncentraciju MP; (4) procjeni ispitivanih vodnih tijela jadranskog sliva kao izvora MP na crnogorskom primoriju i (5) identifikaciji potencijalnih uticaja MP na akvatične organizme, životnu sredinu i zdravlje ljudi.

Predmetna analiza predstavljaće osnov za kreatore legislativa u Crnoj Gori u cilju iznalaženja adekvatnih rješenja za unapređjenje i očuvanje životne sredine, kao i poštovanje principa održivog razvoja.

Područje istraživanja

U ovoj doktorskoj disertaciji, po prvi put, ispitivan je sadržaj MP u površinskim obalnim sedimentima rijeka Zete, Morače i Bojane i Skadarskog jezera koji pripadaju slivu Jadranskog mora u cilju ekološke procjene i dobijanja novih saznanja.

Zeta se nalazi u centralnom regionu Crne Gore, dužina rijeke iznosi oko 89 km, a površina sliva 1547 km² (Sekulić, 2020), protiče kroz Nikšić, Danilovgrad i Podgoricu. Prije izgradnje hidroenergetskog sistema, Zeta je kroz svoj prirodni ponor Slivlje u Nikšićkom polju tekla kao ponornica, u dužini od oko 5 km i ponovo izbijala kao izvor na vrelu Perućice i Glave Zete. Zeta prima nekoliko pritoka, u nju se uliva veći broj stalnih i povremenih potoka, a duž korita Zete izbija veći broj vrela. Uliva se u rijeku Moraču i predstavlja desnu i glavnu pritoku Morače.

Morača je najduža crnogorska rijeka jadranskog sliva i najveća pritoka Skadarskog jezera, dužina rijeke je 113.4 km, a površina sliva 2628 km² (Kračun-Kolarević et al., 2020) i protiče kroz Kolašin, Podgoricu i Cetinje. U gornjem toku Morača je brza planinska rijeka bujičnog karaktera i teče kroz kanjon Platije dug 38 km. Morača prima nekoliko pritoka, od kojih su najveće Zeta (desna pritoka) i Cijevna (lijeva pritoka).

Bojana izvire iz Skadarskog jezera, kod grada Skadar u Albaniji. Najvećim dijelom Bojana je granična rijeka između Crne Gore i Albanije, duga je 41 km od čega Albaniji pripada oko 17.5 km (Barović et al., 2021). Na teritoriji Albanije, u Bojanu se ulivaju rijeke Kiri i Drim. Bojana se uliva u Jadransko more, najveća je pritoka Jadranskog mora u Crnoj Gori, druga najznačajnija pritoka Jadranskog mora poslije rijeke Po i treća rijeka po količini vode koja se uliva u Sredozemno more poslije rijeka Nil i Po (Barović et al., 2021).

Skadarsko jezero je najveće jezero na Balkanskom poluostrvu koje dijele Crna Gora (65 %) i Albanija (35 %). Dužina Skadarskog jezera iznosi oko 44 km, širina oko 14 km, dok površina varira između 353 i 500 km^2 (Pešić et al., 2020). Sliv Skadarskog jezera obuhvata 5631 km^2, od čega oko 81 % pripada teritoriji Crne Gore. Najznačajnija pritoka Skadarskog jezera, koja doprinosi sa 63 % ukupnog dotoka vode u jezero, je Morača (Barović et al., 2018). Skadarsko jezero pripada protočnom tipu jezera, pa vodu ispušta preko rijeke Bojane u Albaniji koja se uliva u Jadransko more u Crnoj Gori.

Eksperimentalni dio

Eksperimentalni dio disertacije sastojao se iz uzorkovanja, pripreme uzoraka, ekstrakcije MP iz uzoraka (sušenje, razdvajanje gustine, razgradnja organske materije, prosijavanja, filtriranja, inkubacije), vizuelne identifikacije (određivanje broja, veličine, boje i oblika MP, inkubacija) i hemijske identifikacije (određivanje tipa polimera). Vizuelna identifikacija je izvršena primjenom STEBD optičkog profesionalnog mikroskopa, a hemijka identifikacija primjenom infracrvene spektrometrije sa Fourier-ovom transformacijom (FTIR). Nakon eksperimentalne analize, dobijeni podaci su statistički obrađeni i izvršena je procjena ekološkog rizika.

Postupci pripreme, ekstrakcije i vizuelne identifikacije uzoraka sedimenta za analizu MP obavljeni su u laboratorijama Metalurško–tehnološkog fakulteta Univerziteta Crne Gore u Podgorici, dok je hemijska identifikacija MP u uzorcima sedimenta obavljena u laboratorijama Morske biološke postaje Nacionalnog instituta za biologiju u Sloveniji u Piranu.

Uzorkovanje površinskog obalnog sedimenta rijeka i jezera vršeno je u dva periodična ciklusa, jesenjem (2022) i proljećnom (2023). Na ispitivanim vodnim tijelima izabrano je 4 do 6 lokacija za uzorkovanje na osnovu specifičnih karakteristika terena, različitog geografskog položaja, mogućnosti pristupa istim, kao i različitih antropogenih aktivnosti u njihovoj neposrednoj blizini. Izabrane lokacija za uzorkovanje obalnog sedimenta rijeka mogu se podijeliti u tri grupe: izvor, sredina toka rijeke i ušće rijeke u drugu rijeku, jezero ili more, dok su lokacije izabrane za uzorkovanje obalnog površinskog sedimenta jezera obuhvatile sve strane Skadarskog jezera, sa različitim stepenom antropogenog uticaja, a koje pripadaju teritoriji Crne Gore.

Analiza glavnih koordinata (PCO), klasterska analiza (CA) i permutaciona multivarijantna analiza (PERMANOVA) korišćene su statističke metode za obradu rezultata. PCO i CA analizom je izvršena karakterizacija uzoraka, tj. identifikovano je grupisanje uzoraka u odnosu na područje, lokacije i sezonu uzorkovanja, dok su na osnovu PERMANOVE izvršena particionisanja promjenljivih faktora kao i procjena uticaja glavnih faktora.

Procjena potencijalnog ekološkog rizika po životnu sredinu i zdravlje čovjeka od identifikovanih polimera u ispitivanim uzorcima sedimenta provjerena je od strane Evropske hemijske agencije (ECHA), a na osnovu zastupljenosti MP u ispitivanim uzorcima sedimenta primjenom indeksa opterećenja MP (PLI) i indeksa polimerne opasnosti (PHI).

Rezultati i diskusija

U svim ispitivanim uzorcima obalnog sedimenta rijeka Zete, Morače i Bojane i Skadarskog jezera identifikovana je MP. Zastupljenost MP u obalnim sedimentima ispitivanih vodnih tijela jadranskog sliva kretala se sledećim nizom: Bojana (180 ± 53.5 MP/kg suvog sedimenta) > Morača (169 ± 113 MP/kg suvog sedimenta) > Skadarsko jezero (153.4 ± 42.7 MP/kg suvog sedimenta) > Zeta (145 ± 110 MP/kg suvog sedimenta). Ukupna srednja zastupljenost MP tokom cijelog istraživanja u ispitivanim rijekama i jezeru jadranskog sliva iznosila je 160.5 ± 83.3 MP/kg suvog sedimenta. CA analiza, PERMANOVA i Monte Carlo test ukazuju da nema značajne statističke korelacije u nivou zastupljenosti MP u odnosu na ispitivana vodna tijela, lokacije i sezonu uzorkovanja ($p > 0.05$). Na osnovu poređenja sa literaturnim podacima, ispitivane rijeke i jezero su srednje zagađeni MP. Veća zastupljenost MP u proljećnoj sezoni uzorkovanja može biti posledica većeg priliva kopnenih voda, dok veća zastupljenost MP u jesenjoj sezoni uzorkovanja može biti posledica većih antropogenih uticaja i aktivnosti tokom ljetnje sezone, što je u skladu sa studijom Zeri i sar. (2018). Ukupna srednja zastupljenost MP identifikovana u sedimentima rijeka Zete, Morače i Bojane i Skadarskog jezera bila je u skladu sa literaturnim podacima iz regiona i širom svijeta. Dobijeni rezultati su opravdali očekivane, jer reflektuju različite antropogene uticaje na ispitivanim vodnim tijelima. Zastupljenost MP u rječnim sedimentima u blizini urbanih područja kao i u blizini ušća rijeka ukazuje da je gustina naseljenosti kritičan faktor koji utiče na distribuciju MP, što je u skladu sa prethodnim studijama (Xu et al, 2020; Firdaus et al, 2020). Područje istraživanja obuhvata centralni region (Nikšić, Danilovgrad, Podgorica i Cetinje) i primorski region (Bar i Ulcinj), koji zajedno pripadaju jadranskom slivu Crne Gore. U navedenom području se nalaze četiri postrojenja za prečišćavanje otpadnih voda (PPOV), a koja karakteriše nesklad između razvoja kolektorskog sistema, raspoloživosti i kapaciteta. Ova studija ukazuje na indirektan uticaj rijeka Zete i Morače i Skadarskog jezera, te direktan uticaj rijeke Bojane na zastupljenost MP na Crnogorskom primorju.

Zastupljenost oblika MP u obalnim sedimentima svih ispitivanih vodnih tijela jadranskog sliva (Zeta, Morača, Bojana i Skadarsko jezero) kretala se sledećim nizom: vlakna > fragmenti > filmovi > granule. U ispitivanim uzorcima sedimenata vlakna, praćena fragmentima su bila najzastupljeniji oblik MP, dok granule nisu identifikovane u sedimentima Skadarskog jezera. Dobijeni rezultati su u skladu sa prethodnim studijama koje su se bavile ispitivanjem zastupljenosti oblika MP u riječnim i jezerskim sedimentima (Vermaire et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon–Sanchez et al., 2019; Turner et al., 2019; Egessa et al., 2019; Felismino et al., 2021; Matjašič et al., 2022). Hernandez i sar. (2017) navode da su izvori vlakna u akvatičnim ekosistemima uglavnom: ispusti otpadnih voda, sa naglaskom na otpadne vode iz mašina za pranje veša; oprema za ribolov i robolovne aktivnosti, kao i tekstilna industrija. Dok fragmenati u akvatičnim ekosistemima najčešće potiču kao rezultati degradacije čvrste makro i mezo plastike na plastiku opsega veličine MP (Wang et al., 2016). PCO analiza, PERMANOVA i Monte Carlo test ukazuju da nema značajne statističke korelacije u nivou zastupljenosti tipa oblika MP u odnosu na ispitivana vodna tijela i sezonu uzorkovanja ($p > 0.05$).

Sve identifikovane čestice MP u uzorcima obalnih sedimenata rijeka i jezera bile su u opsegu veličine MP (0.1–5 mm). Zastupljenost veličine MP u obalnim sedimentima svih ispitivanih vodnih tijela jadranskog sliva (Zeta, Morača, Bojana i Skadarsko jezero) kretala se sledećim nizom: 0.5–1 mm > 1–3 mm > 3–5 mm > 0.1–0.5 mm. Rezultati ukazuju da je MP srednje kategorije veličine najzastupljenija u ispitivanim uzorcima sedimenata, što je u skladu sa podacima iz literature za MP identifikovanu u sedimentima rijeka i jezera (Abidli et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon–Sanchez et al., 2019; Turner et al., 2019; Egessa et al., 2019; Felismino et al., 2021; Matjašič et al., 2022). PCO analizom, PERMANOVOM i Monte Carlo testom se uočava statistička korelacija zastupljenosti veličine MP u odnosu na sezonu uzorkovanja ($p < 0.05$), dok se ne uočava statistička korelacija zastupljenosti veličine MP u odnosu na ispitivana vodna tijela ($p > 0.05$).

Zastupljenost boje MP u obalnim sedimentima svih ispitivanih vodnih tijela jadranskog sliva (Zeta, Morača, Bojana i Skadarsko jezero) kretala se sledećim nizom: plava > providna >

crvena > crna > žuta > zelena > bijela. Plava, providna i crvena su najzastupljenije kategorije boja u ispitivanim uzorcima sedimenata. Navedeni rezultati su u skladu sa prethodnim studijama koje su ispitivale zastupljenost boje MP u rječnim i jezerskim sedimentima (Corocran et al., 2015; Abidli et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon–Sanchez et al., 2019; Turner et al., 2019; Felismino et al., 2021; Matjašič et al., 2022). Abidli i sar. (2017) ukazuju da plava i providna boja MP može ukazivati da plastika u akvatičnim ekosistemima potiče od degradacije plastičnih boca, folija, kesa, omota i sl., odnosno može ukazivati da identifikovana MP potiče od ambalažnog otpada. PCO analiza, PERMANOVA i Monte Carlo test ukazuju da nema značajne statističke korelacije u nivou zastupljenosti boja MP u odnosu na ispitivana vodna tijela i sezonu uzorkovanja ($p > 0.05$).

Od ukupno 642 vizuelno identifikovane čestice MP tokom čitavog istraživanja, 28 % je hemijski identifikovano, od čega 13.2 % pomoću ATR FTIR i 14.8 % pomoću µFTIR. Čestice MP koje su odabrane za hemijsku analizu su čestice različitog oblika, boje i veličine iz svakog pojedinačnog uzorka. U uzorcima površinskih obalnih sedimenata rijeka i jezera FTIR–om su hemijski identifikovani sljedeći polimeri: polipropilen (PP), polietilen (PE), polietilen tereftalat (PET), poliamid (PA), polistiren (PS), polivinil hlorid (PVC), akrilat kopolimer (Acrilat cop.), polivinil alkohol (PVA) i politetrafluoroetilen (PTFE). PP je bio najzastupljeniji tip polimera u sedimentima rijeke Zete i Bojane, dok je PE bio najzastupljeniji tip polimera u sedimentima rijeke Morače i Skadarskog jezera, što je u skladu sa prethodnim studijama koje su se bavile ispitivanjem zastupljenosti tipova polimera MP u rječnim i jezerskim sedimentima (Klein et al., 2015; Rodrigues et al., 2018; He et al., 2019; Simon–Sanchez et al., 2019; Constant et al., 2020; Matjašič et al., 2022). Yuan i sar. (2019) ukazuju da rezultati hemijske identifikacije polimera MP mogu pomoći da se identifikuje trag izvornom obliku plastičnih ostataka u životnoj sredini. PE i PP su prijavljeni u literaturi kao dva tipa polimera sa najvećim globalnim obimom proizvodnje i upotrebe, velikom distribucijom, a samim tim i većom vjerovatnoćom da postanu plastični otpad u slatkovodnim ekosistemima (Plastics Europe, 2021). PCO analiza, PERMANOVA i Monte Carlo test ukazuju da nema značajne statističke korelacije u nivou zastupljenosti tipova polimera MP u odnosu na ispitivana vodna tijela i sezonu uzorkovanja ($p > 0.05$).

ECHA je klasifikovala potencijalne opasnosti od identifikovanih polimera u površinskim obalnim sedimentima rijeka i jezera po zdravlje ljudi, akvatične organizme i životnu sredinu na sledeći način: PP, PE, PET i PTFE neopasni, PA i PVC opasni, a PS, Acrilat cop. i PVA sa signalima upozorenja. ECHA klasifikacija je zasnovana na hemijskom sastavu čistih „djevičanskih“ polimera. Najzastupljeniji polimeri u ovoj studiji (PP, PE i PET), klasifikovani su kao neopasni prema ECHA, međutim ne treba zanemariti činjenicu da se u industriji plastike koristi širok spektar aditiva sa različitim svojstvima koji se mogu osloboditi iz plastike tokom njenog životnog ciklusa što dovodi do izloženosti ljudi i životne sredine.

Rezultati indeksa opterećenja MP (PLI) su ispitivane sedimente rijeka i jezera na svakoj ispitivanoj lokaciji posebno, kao i u rijekama i jezeru tokom cijelog perioda istraživanja ukupno, klasifikovali u kategoriju I. Prema vrijednostima PLI, sedimenti rijeka Zete, Morače i Bojane i Skadarskog jezera su neznatno kontaminirani MP. Nasuprot navedenim rezultatima PLI, poređenjem sa dostupnim literaturnim podacima o zastupljenosti MP u slatkovodnim ekosistemima, ispitivani sedimenti u ovoj studiji su srednje do visoko zagađeni MP, u zavisnosti od ispitivane lokacije. Slični rezultati dobijeni su u studijama koje su se bavile procjenom vrijednosti PLI u slatkovodnim ekosistemima (Wang et al., 2021; Warrier et al., 2022; Amrutha et al., 2023;). Sve u svemu, manje vrijednosti PLI (< 10) pronađene u ispitivanim rijekama i jezeru u ovoj studiji su posledice relativno visokih pozadinskih vrijednosti MP.

Na osnovu vrijednosti indeksa polimerne opasnosti (PHI), ukupan rizik od zagađenja MP u sedimentu rijeke Zete je klasifikovan kao nivo opasnosti IV (100–1000), dok su ukupni rizici od zagađenja MP u sedimentima rijeka Morače i Bojane i Skadarskom jezeru klasifikovani kao nivo opasnosti V (> 1000). Vrijednosti PHI ukazuju na ozbiljan trend zagađenja MP. Visoke PHI vrijednosti su posledice visokog prisustva MP sa visokim ocjenama opasnosti, kao što su Acrilat cop., PA i PS. Slična zapažanja iznijeli su i Amrutha i sar. (2023), Kasamesiri i sar. (2023) i Ranjani i sar. (2021) u svojim studijama.

Zaključci

Rezultati doktorske disertacije ukazuju da ispitivani slatkovodni ekosistemi nisu samo putevi emisije MP sa kopna u mora i okeane, već i sekundarni izvori i rezervoari prethodno akumulirane MP. Ova studija ukazuje da se identifikovane vizuelne i hemijske karakteristike MP ne razlikuju između slatkovodnih ekosistema u ovoj studiji i morskih ekosistema u ranije ispitivanim studijama u Crnoj Gori (Bošković et al., 2021, 2022a, 2022b, 2022c, 2023), pa se može zaključiti da priliv kopnenih voda iz jadranskog sliva doprinosi povećanju zastupljenosti MP na crnogorskom primoriju. Ovi nalazi naglašavaju hitnost daljeg praćenja slatkovodnih ekosistema i

identifikovanja tačkastih izvora za ublažavanje MP kontaminacije akvatičnih ekosistema u bliskoj budućnosti.

Rezultati ove studije ukazuju da identifikovana MP vodi porijeklo od otpadnih voda i fragmentacije većih plastičnih ostataka što ukazuje na veliku upotrebu plastike i njeno neadekvatno odlaganje od strane stanovništva Crne Gore. Stoga je kontrola plastike/MP na izvoru opcija kojoj treba ozbiljno posvetiti pažnju. Zakonske regulative i odluke na lokalnom i nacionalnom nivou u pogledu smanjenja upotrebe plastike, naročito plastike za jednokratnu upotrebu i plastične ambalaže su ključne u smanjenju i rješavanju problema zagađenja plastikom/MP. Vlada Crne Gore treba da usklađenim naporima optimizuje i poboljša procese i upravljanje PPOV i upravljanje otpadom na nivou Crne Gore, što je prije moguće.

Ova studija daje prve informacije o stanju, izvorima i ekološkom riziku zagađenja MP u ispitivanim slatkovodnim ekosistemima Crne Gore. Potrebne su dalje studije o vremenskim varijacijama zagađenja MP i ekološkog rizika MP kako bi se unaprijedilo znanje o sudbini, transportu i uticajima MP na životnu sredinu i zdravlje čovjeka. Takođe, predlaže se konstantan monitoring uticaja i sudbine MP u slatkovodnim ekosistemima, kao i procjena potencijalnih uticaja na ljude koji proističu iz konzumiranja ribljih proizvoda.

Ključne riječi: mikroplastika, jadranski sliv, sediment, rijeke, jezero, Crna Gora
Naučna oblast: održivi razvoj, zaštita životne sredine
Uža naučna oblast: održivi razvoj, zaštita životne sredine

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Figure 6.6 PCO and CA analysis of MP polymer type abundance in sediment samples of river and lake during the entire research period

CONTENT

INTRODUCTION

The title of the dissertation „*Contamination of freshwater ecosystems of Montenegro with microplastics: First observations on occurrence, abundance, spatial patterns, identification and ecological assessment*“ refers to the ecological condition and quality of selected freshwater ecosystems (rivers and lake) in Montenegro regarding the content of microplastics in inshore sediments as important ecosystem units.

The world is often faced with increasing amount of environmental pollution, especially of aquatic ecosystems, and plastics, therefore microplastics, are counted among the pollutants of global proportions. The abundance and permanence of microplastics (plastic particles smaller than 5 mm) are recognized problems that have been occurring in aquatic ecosystems for the last 15 years (Andrady, 2011; Cole et al., 2011). There is no part of the Planet where microplastics have not been identified: on the surface of the Earth, at great depths in the seas and oceans (Woodall et al., 2014), in rivers and lakes (Rios Mendoza and Balcer, 2018), in the aquatic organisms digestive tract (Andrady, 2011), in polar ice (Tekman et al., 2020), in the atmosphere (Gasperi et al., 2018), on the highest mountain peaks (Bergmann et al., 2019), in numerous food sources for human consumption (honey, seafood, fish, sea salt, poultry, bottled water, tap water, sugar, beer, etc.) (Toussaint et al., 2019), and even in the placenta of newborn babies (Ragusa et al., 2021).

A greater number of studies indicate the presence of microplastics in marine ecosystems, and a smaller number of studies testify to the presence and impact of microplastics in freshwater ecosystems (Moore et al., 2011; Fahrenfeld et al., 2019). Today, the number of studies dealing with the identification of plastic residues within freshwater ecosystems, i.e. the sources of microplastics, their ways of reaching freshwater ecosystems and groundwater, as well as potential impacts on freshwater ecosystems and human health, is increasing (Eriksen et al., 2013; Scherer et al., 2017; Turner et al., 2019; Lončarski, 2020).

Studies indicate that freshwater ecosystems have an important influence in the transport of microplastics. Freshwater is considered one of the significant sources of microplastics in the oceans and seas and the significant transport vector of plastic pollution from land sources (Turner et al., 2019; Iannilli et al., 2020; Dusaucy et al., 2021), so the study of freshwater ecosystems on plastic/microplastic presence is of great importance (Dusaucy et al., 2021). The microplastic abundance in freshwater ecosystems endangers the environment, given that aquatic organisms can be ingested microplastics, harming their health, affecting aquatic biodiversity and humans as the last link in the food chain (Horton et al., 2017).

Lakes represent sinks for microplastic waste compared to the oceans, which are subject to global long-range and transport from several basins (Hidalgo-Ruz et al., 2012). Studies indicate that rivers and river sediments are significant sources of microplastics in marine ecosystems (Peng et al., 2018). As surface sediments are considered the ultimate repository of microplastics, sediments are very good indicators of pollution as they testify to the historical incorporation of microplastics (Turner et al., 2019).

Until recently in Montenegro, examining the microplastics presence in the environment was not a subject of interest and study, so there were no data and knowledge on the topic. Today, there are significant scientific contributions in examining the microplastics presence in the marine ecosystem of the Montenegrin coast (Bošković et al., 2022b, 2022c, 2023), but until this study, there were no studies on the microplastics presents in Montenegrin freshwater ecosystems.

Bearing in mind the above, this dissertation aims to comprehensively, for the first time: evaluate the level of abundance, movement dynamics, source characterization and fate of microplastics in the Zeta, Morača and Bojana rivers of the Adriatic basin and Skadar lake; to assess the pollution of the examined localities from the point of view of the microplastics abundance in surface inshore sediments; assesses the impact of the examined water bodies of the Adriatic basin as a source of microplastics on the Montenegrin coast and identifies the potential impacts of microplastics on aquatic organisms, the environment and human health. The subject analysis will represent the basis for the creators of legislation in Montenegro with the aim of finding adequate solutions for the improvement and preservation of the environment, as well as respecting the principles of sustainable development.

THEORETICAL PART

1. PLASTICS

1.1 Advantages and Production

Because products of plastic are used worldwide and their production has increased dramatically since their initial commercial development in the 1950s (Plastics Europe, 2008), the "*plastic age*" is called the current period of the human history (Thompson et al., 2004; Cozar et al., 2014; Yang et al., 2020). Plastics are an ideal material for various purposes, characterized by its low price, light weight, easy production, high mechanical strength, moisture resistance, corrosion resistance, easy to shape, durability, etc. (Van Cauvenberghe et al., 2015; Yang et al., 2020). Because of its qualities, plastics have replaced natural materials that were used until now like: glass, metal, leather, wood and stone (Rios mendoza and Balcer, 2018; Emmerik and Schwarz, 2019). Plastics is important in our society and provides a number of advantages with positive effect on human health and environment:

- The use of plastic as packaging has resulted in a high level of food preservation, an increase in shelf life, facilitating the possibility of transportation and saving resources (Emmerik and Schwarz, 2019).
- Plastic is use in the transportation sector (plastic packaging), compared to other materials, reduces the fuel consumption and results in high reduction of emissions of CO_2 (Palencia et al., 2012).
- Plastic water supply systems provide clean water (Plastics Europe, 2008).
- Personal protective clothes and safety equipment prevent injuries at work (Hahladakis et al., 2018).
- Plastic products have found wide use in medicine and health care, contributing to safer work (Andrady i Neal, 2009).
- Also, plastics are used in construction, transportation, sports and leisure, electronics, agriculture, cosmetics, design and production and many other aspects of the society making human lives more comfortable (FAO, 2017; Egessa et al., 2019).

Around 30.000 polymer materials have been registered in European Union to date and 75 % of the total demand for plastics is limited to several types of polymer materials: polyethylene

(PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (Plastics Europe, 2021; Bellasi et al., 2020).

The popularity of plastics is growing significantly and its production is still increasing, Figure 1.1. Global plastics production has increased to approximately 400 million tons per year (Plastics Europe, 2021).

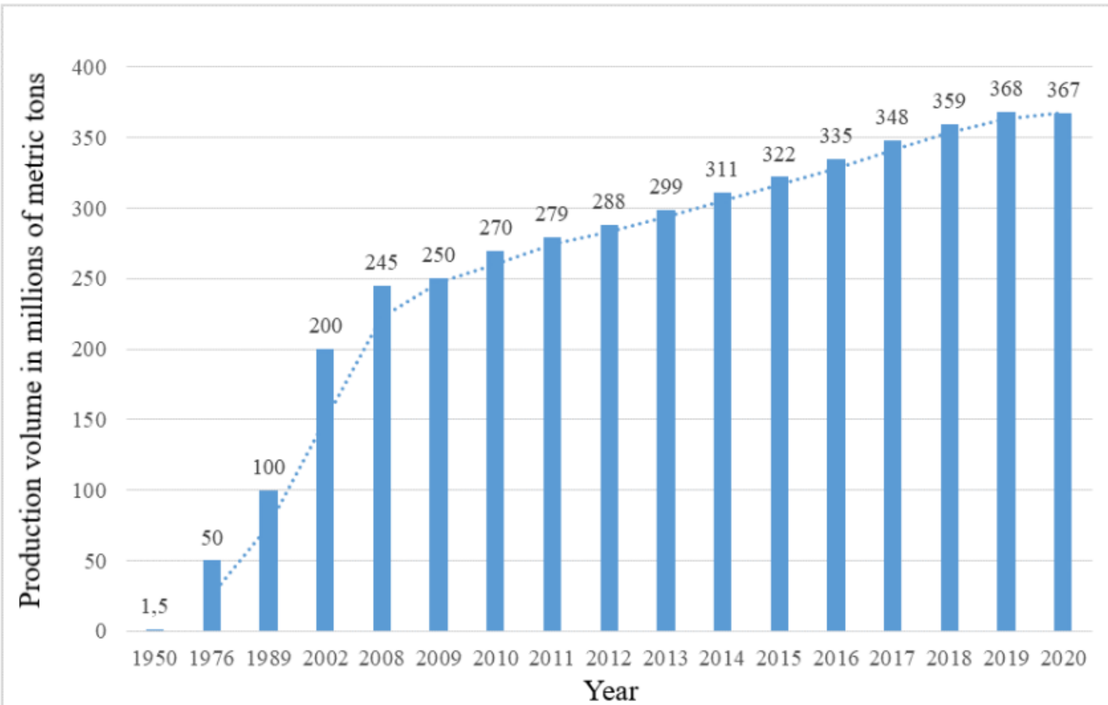


Figure 1.1 Projection of plastic production worldwide from 1950 to 2020 (Plastics Europe, 2021)

Plastics (from the Greek "plastikos" which means it can be shaped), is made of organic synthetic polymers which are usually produced by polymerization of the monomers obtained from natural gas, coal or crude oil, more precisely from resources and reserves of fossil fuels (Ivleva et al., 2017). Plastics can also be produced from other, more ecologically acceptable sources that replace fossil fuels. Plastics known as renewable plastics or bioplastics are made from renewable biomass such as carbohydrates, starches, vegetable fats, oils, terpenes, lignin, cellulose, wood fibers, recycled food waste (Wiesinger et al., 2021). Biodegradable plastic does not contain potentially dangerous chemicals, but its lifespan is shorter. The largest amount of produced plastic is synthetic. However, limited reserves of fossil fuels drive the need for greater production of plastic from renewable sources. More sustainable alternatives to conventional plastics should be considered which may include bio-based plastics and composite plastics that have a low impact on environment (Spierling et al., 2018). The use of bio-based materials as an alternative to plastic promotes the formation of a circular economy and has numerous advantages such as possibility of recyclability/biodegradability, biocompatibility and relatively low environmental toxicity (Teixeira-Costa and Andrade, 2021; Wiesinger et al., 2021).

1.2 Composition of Plastics

Various chemical substances are used in the production and processing of plastics (Hahladakis et al., 2018). The basis of plastics is made of organic polymer matrices, more precisely monomeric units that are repeating (Andrady and Neal, 2009). Polymers are chemical compounds whose molecules are very big, like long chains, formed by many units (monomers) joined by covalent bonds that build polymers. The structure and length of these chains dictate the properties of the resulting plastic. Polymers can be divided into two categories depending on the chemical structure of the molecules: aliphatic and aromatic polymers. Polymers usually contain atoms of carbon, hydrogen, oxygen, nitrogen and sulfur in their structure. In many plastic products, the polymer is just one ingredient, Figure 1.2. In order to achieve a set of properties appropriate to the product, polymers are almost always combined with other ingredients (additives) that are added during the processing and production of the desired plastic products (Wiesinger et al., 2021).

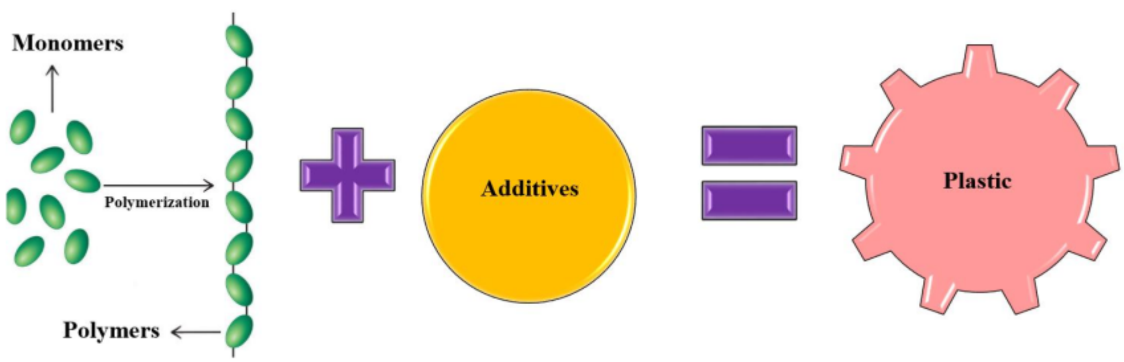


Figure 1.2 Composition of plastics

1.2.1 Additives in Plastics

Plastics have a wide range of applications, therefore it is necessary to add additives during the production of plastics in order to adapt the properties of the polymer to the desired end product (Marturano et al., 2017). Additives are chemical compounds that are added in the process of production of plastics in order to improve the performance, functionality and properties of aging of the polymers. Each of the additives has a special role in delivering/improving the (final) functional properties of the plastic product (Hansen et al., 2013). In the plastics industry are used a wide range of additives which give different properties to the final product (Hahladakis et al., 2018):

- Functional additives (plasticizers, stabilizers, flame retardants, slip agents, antioxidants, antistatic agents, lubricants, hardening agents, biocides, etc.);
- Colorants (azocolorants, pigments, soluble, etc.);
- Fillers (talc, kaolin, clay, calcium carbonate, barium sulfate, etc.);
- Reinforcements (glass fibers, carbon fibers, etc.).

The most commonly used functional additives are plasticizers. Plasticizers manipulate the flexibility, stretchability and workability of plastics, making it suitable for the target final product (Cano et al., 2002; Marturano et al., 2017; Jamarani et al., 2018). Stabilizers are functional types of additives that are used to prevent degradation of material by oxidation or different weather conditions in order to extend the life of the product (Kirschveng et al., 2017). Thermal stabilizers prevent thermal degradation of polymers when they are exposed to elevated temperatures. Since plastics are a carbon-based materials that can potentially catch fire, flame retardants are often used in the industry of plastics. Which type of flame retardant will be used depends on a wide range of factors such as the type of polymer and the test risk scenario (Morgan and Gilman, 2013). Slip additives are commonly used to reduce static charge, which is especially useful for thin films (Lau and Wong, 2000). Slip additives contribute to the reduction of the surface coefficient of friction of the polymer, enable better release from the mold, reduced melt viscosity and anti-sticking properties (Bhunia et al., 2013). Highly reactive free radicals produced by heat and radiation lead to polymer degradation. Antioxidants are incorporated into various polymer resins in order to reduce the oxidative degradation of plastics if/when exposed to ultraviolet (UV) radiation (Sablani and Rahman, 2007; Bhunia et al., 2013). Flame retardants are used to prevent ignition or spread of flame in plastic material. Plastics have significant uses in constructions, electrical and transportation applications that must meet fire safety standards (Sablani and Rahman, 2007). Colorants as types of additives are used to obtain the desired color of plastics, and therefore act in aesthetic aspects (Hahladakis et al., 2018). Another frequently used type of additive are fillers. Various properties and physical aspects of the polymer can be changed depending on the type of filler used in the production of the plastic. Reinforces are used to improve the mechanical properties of plastics (Bart, 2005).

Table 1.1 presents examples of the most frequently used additives in plastics, with the highest percentage of abundance, as well as their potential risks to the environment and human health. The potential risks to the environment and human health from the most commonly used additives in plastic materials have been checked by the European Chemical Agency - ECHA (www.echa.europa.eu/home). Impacts on the environment and human health are based on Harmonized classification and labeling - CLH, which is harmonized with the European Commission EC No 1907/2006) of the European Parliament and the Council of the Registration, Evaluation, Authorization and Restriction of Chemicals - REACH).

Additives with known negative effects, which are incorporated during the production of plastics, can have a negative impact on human health and the environment due to their release (Bellasi et al., 2020). Additives can be released from plastics during the life cycle of the plastic which leads to human and environmental exposure (Velis, 2015; Hahladakis et al., 2018). Growing scientific evidence and public concern regarding the toxicity of additives used in the production of plastics have led to finding regulatory measures focused on limiting the use of dangerous and harmful additives in plastics (Plastics Europe, 2021).

Table 1.1 Overview of the impact on health and the environment of the most commonly used additives in plastic materials, with the highest percentage of abundance (www.echa.europa.eu/home)

Additive types	Most commonly used substances	EC / list number	Hazard classification and labeling by CHA i CLP**
1. Functional additives			
Plasticizers (10-70)*	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich (DIHP)	276-158-1	According to CLP (ATP01 for DIHP and ATP17 for boric acid) the listed substances can harm fertility and can have a negative effect on the fetus. Substances predicted to meet the criteria for carcinogenicity, mutagenicity or reproductive toxicity category 1A or 1B, or which are predicted to meet the criteria for the classification of health or environmental hazards.***
Flame retardants (3–25)*	Boric acid	234-343-4	
Stabilizers (0.5–3)*	Lead and lead compounds	231-100-4	According to the ECHA classification in REACH registrations, these substances can affect fertility, as well as the fetus itself. They cause organ damage through prolonged or repeated exposure, are highly toxic to aquatic life with long-lasting effects, can cause cancer, are highly toxic to aquatic life and can harm nursing infants. Some submitters report this substance as carcinogenic. ***
2. Colorants (0.01–10)*	Cadmium and cadmium compounds	231-152-8	According to the ECHA classification in REACH registrations, these substances are lethal if inhaled, are highly toxic to aquatic life, can cause cancer, cause organ damage through prolonged or repeated exposure, are suspected of causing genetic defects, are suspected of harming fertility and fetus. They can ignite spontaneously if exposed to air. These substances are carcinogenic, suspected to be mutagenic and suspected to be toxic to reproduction. ***
3. Fillers (up to 50)*	Calcium carbonate	207-439-9	According to the ECHA classification in REACH registrations, this substance causes serious eye damage, causes skin irritation and may cause respiratory irritation.***
4. Reinforcements (15–30)*	Carbon fibers	231-153-3	Warning! According to ECHA's classification in CLP notices, this substance causes serious eye irritation, is self-heating in large quantities, can catch fire and can cause respiratory irritation.

* Range of common quantities (% v/v) (Hahladakis et al., 2018)
** Classification, Labelling and Packaging - CLP covers hazardous chemicals
*** Substances of very high concern - SV

1.3 Degradation of Plastics

Plastic is considered a durable material. Most types of plastic polymers are not biodegradable, while non-degradable polymers (known as synthetic plastics) can be degraded/fragmented by various mechanisms: mechanical degradation, hydrolytic degradation, photodegradation and thermo-oxidative degradation (Zeenat et al., 2021).

- ***Biological degradation*** is a complex process of physicochemical transformation of polymers into smaller units through the action of microorganisms (Ghosh et al., 2013). There are six main microorganisms that have the ability to degrade plastic polymers: bacteria, archaea, fungi, protozoa, algae and viruses. Biodegradable polymers are materials that, with the activity of microorganisms, are decomposing, in a limited period of time, into carbon dioxide, water, minerals and biomass (aerobic biodegradation), or into carbon dioxide, methane and humic material (anaerobic biodegradation) (Matjašič et al., 2021). The degree of biodegradability of plastics depends on their chemical and physical properties: surface conditions (hydrophilic and hydrophobic properties); structure (chemical structure and molecular weight), specific characteristics (glass transition temperature, melting temperature, modulus of elasticity, crystallinity and crystal structure) (Tokiwa et al., 2009). A study by the author Matjašič et al. (2021) who dealt with the critical evaluation of study of biodegradation of synthetic plastics through a systematic review of the literature indicates that all tested synthetic polymers show some kind of surface damage or initiation of chemical changes due to the activity of microorganisms. However, these changes are small and slow and reports on them for the various synthetic polymers tested have shown highly inconsistency between publications, necessitating the need for international standardization of tests of biodegradation of synthetic polymers.
- ***Mechanical degradation*** involves the breaking/tearing of polymer chains as a result of mechanical stress (Li et al., 2005). Mechanical degradation refers to the changes in the mass of the structure of polymers as cracking, brittleness and peeling of the plastic/polymer occurs. Plastic can be degraded or eroded due to naturally caused mechanical movements or friction such as waves, wind, hitting rocks, etc. (Zeenat et al., 2021).

- ***Hydrolytic degradation*** occurs in polymers that are sensitive to water, especially those that absorb a lot of moisture which results in the breaking of polymer chains (Zeenat et al., 2021). Plastics absorb water in different degrees, depending on their molecular structure. Plastics must be exposed to moisture and elevated temperature in order to initiate hydrolytic degradation of the polymer (Li et al., 2005).
- ***Photodegradation*** occurs due to long-term exposure to ultraviolet (UV) radiation, during which polymer structures can disintegrate. Synthetic polymers are prone to degradation by processes that are initiated by UV radiation. However, if the supply of radiation is stopped, then the degradation of the polymer is also stopped (Lomonaco et al., 2020). Photodegradation results in the splitting of C–C bonds in the polymer chain (Tyler, 2004). Photodegradation of polymers results in changes at the molecular level, more precisely the creation of new molecules with significantly shorter lengths of chains (Zeenat et al., 2021).
- **Thermo-oxidative degradation** occurs as a consequence of high temperature that acts on the polymer (Teare et al., 2000). At high temperature, a big number of polymers are depolymerized (Awasthi et al., 2017). Thermal degradation heats up molecules and atoms until they start moving fast enough to cause collisions with each other. Oxidative degradation implies the loss of electrons, and can be defined as a chemical process in which a substance gains oxygen or loses electrons and hydrogen. Most polymers are subject to thermo-oxidative aging in the environment, so this degradation mechanism is considered to be the most effective for the degradation of plastics. The impact of thermo-oxidative degradation on polymers depends on their chemical structure, more precisely added additives (Zeenat et al., 2021).

Under aquatic conditions, any degradation is quite slow, which is not surprising given that the primary properties of plastic materials are high stability and durability (Zheng et al., 2005). Therefore, the time frame for complete degradation could be significantly extended and reach, in some cases, even hundreds of years (Webb et al., 2012). The rate of plastic degradation is very low, depending on the type of plastic and conditions of environmental, which leads to the accumulation of plastics in the environment (Lončarski, 2020). The rate of degradation of plastics is considered to be highest on shores, as plastics on shores are exposed to high UV levels of radiation and high temperatures, increasing the rate of degradation (Emmerik and Schwarz, 2019).

The effect of the mentioned mechanisms of degradation on plastics/polymer is brittleness and "splitting" of plastic/polymer into smaller pieces (Webb et al., 2012). After mechanisms of degradations, larger plastic items are considered to be the source of a large amount of smaller plastic items < 5 mm in size known as microplastics (Emmerik and Schwarz, 2019).

1.4 Plastics as an Ecological, Aesthetic, Economic and Social Problem

Plastics have changed human life because it is used in different purposes due to its extraordinary characteristics (Ivleva et al., 2017; Bellasi et al., 2020). However, due to the increased production of plastic materials, their long life span, short period of use of plastic products (it is estimated that approximately 40 % of produced plastic products have period of use shorter than 1 month - packaging) there is an increased amount of plastic waste that creates numerous challenges as well as the possibilities of managing this type of waste (Bellasi et al., 2020). Plastic pollution is distributed from pole to pole, from the Arctic (Kanhai et al., 2018) to the Antarctic (Waller et al., 2017), causing great concern to the social and scientific community. Despite concerns about the implications of plastic waste on environment, consumption of plastics continues to grow due to low production costs and high public demand (Oktavilia et al., 2020)." *The Plastic Age*" creates various ecological, aesthetic, economic and social impacts (Bellasi et al., 2020; Zhang et al., 2016).

The ecological impacts of the "*plastic age*" are reflected in environmental pollution, habitat degradation, negative impact on organisms by disrupting their food chain and affecting biodiversity, the death of individuals, changes in the carbon cycle and global warming due to an increase in concentration of CO_2, impact on human health and quality of life, as well as ecotoxicity (MacLeod et al., 2021).

The aesthetic impacts of the "*plastic age*" are reflected in the building up and accumulating of plastic waste in the environment disrupting the visual aspect and landscape (Adomat and Grischek, 2020).

The economic impacts of the "*plastic age*" are reflected in the damage of infrastructure, industrial equipment, high prices for recycling, incineration, disposal of plastic waste, high costs of the remediation of polluted sites and negative impacts on tourism (Emmerik and Schwarz, 2019). On the other hand, valuable (secondary) materials are lost through discarding plastics such as packaging and disposable items. Approaches to solving resource problems from a waste perspective are basically based on reuse and recycling. By reusing and recycling, it is possible to maintain the material and functional value of plastic. Reuse extends the life of a material or product, while recycling plastic is considered a valuable secondary source of material. This way of managing this type of material would significantly reduce the generation of waste, as well as the need for the production of new plastic, and at the same time, the value of plastic is best preserved and additional costs are avoided (Wagner, 2021). The main goal of economic solutions in terms of managing the problem of plastic pollution is to reduce the consumption of plastic at the source. The above can be implemented through financial incentives or through the creation of equal conditions for other solutions, which includes the application of alternative materials that replace plastic and recycling through circular economy models. One of the most effective and at the same time the cheapest economic instruments is based on the introduction of additional taxes on disposable products. It is considered that the price increase would significantly affect the reduced consumption of plastic products (European Council, 2020; Simon, 2020).

The social impacts of the "*plastic age*" include a synergy of aesthetic, ecological and economic impacts. In urban areas, plastic remains can lead to clogging of the drainage hydraulic infrastructure and blocking of the urban drainage systems increasing the risk of floods (Njeru, 2006; Windsor et al., 2019). In addition to the mentioned, plastic waste also affects people's health when they ingest and/or inhale plastics/microplastics (Zhang et al., 2016). On the other hand, plastic consumption behavior is conditioned by numerous factors, among which are convenience, sociodemographic variables, habits, social factors and attitudes towards the environment (Heidbreder et al., 2019). Prohibition of use of plastic packaging for single use like: dishes, straws, bags and cutlery, the convenience and habits of consumers reducing their choice. The prohibition of use of the plastic bags is now in effect in several countries, and prohibition of other products for single use are also in effect (Schnurr et al., 2018). The impact on changing social behavior can come from politics by applying measures such as bans and taxes, but also by implementing a wider public awareness campaign about the harmfulness of plastics including information campaigns, educational programs, interventions when buying (for example asking if customers want plastic bags instead sharing them without asking) and participating in cleaning activities (Heidbreder et al., 2019; Pahl et al., 2020), which is in line with the idea that conscientiousness about plastic pollution is a gateway to broader pro-environmental attitudes (Ives, 2017).

Plastics are designed to last because of their unique molecular structures, and inappropriately disposed plastic items are accumulate in the environment and are present in every section of the environment (Andrady, 2017; Emmerik and Schwarz, 2019; Bellasi et al., 2020). It is estimated that 79 % of produced plastic is either disposed in landfills or enters the natural environment, where plastic accumulates and persists over a long period of time, 12 % is incinerated and 9 % is recycled (UNEP, 2021). Disposal of plastics is expensive and the worst ecological way of managing this type of waste. Disposed plastics will degrade very slowly. On the other hand, incineration of plastics releases toxins such as dioxins (Verma et al., 2016; Wu et al., 2021). According to the World Health Organization - WHO, dioxins are recognized carcinogens (WHO, 2016). Recycling is an expensive way to manage plastics because the composition of plastics can be very diverse which requires each type of plastics to be recycled through a different process (Monroe, 2014). Some strategies of management of plastic pollution are linked to the phase of consumption (e.g. through taxes on products, to reduce generation of waste) and to the phases of management of waste (e.g. improving collection, control of landfills) (Borrelle et al., 2020). The use of alternative solutions, such as bioplastics, has also become increasingly popular in recent years. Due to the complexity of the production and use of bioplastics, clear policies and scientific findings regarding their application are still under development. Bio-based plastics can be degraded in a shorter period of time, and the most convenient method of disposal is composting (Bharadwaj and Shreishi, 2015).

Lack of ecological awareness and inability to manage plastic/MP waste efficiently and prevent further emissions of plastics/MP into environment are the main cause of the global problem of plastic pollution. Hence, plastic pollution at its core becomes engineering problem that could be treated with a number of technological solutions (Wingfeld and Lim, 2021). Activities like cleaning of rivers, beaches, lakes, on the open ocean, etc., could be considered as a part of the set of solutions for the problem of waste. Removing plastic remains from the environment can go from low technology solutions that include citizens that clean polluted environment (Ocean Conservancy, the Nordic Coastal Cleanup), across medium technology solutions that refer to collecting remains before they reach oceans and seas (Mr. Trash Wheel, the Great Bubble Barrier), to high technology solutions developed by Ocean Cleanup or remotely operated underwater vehicles (Schmaltz et al., 2020; Wingfeld and Lim, 2021). Cleanup activities can significantly increase awareness of pollution and promote the importance of environmental protection with more sustainable changes in society as a whole (Wiles et al., 2017, 2019), so engaging and empowering volunteers is a very important step.

Initiatives at the global level are based on the reduction of plastic pollution of the environment and its rehabilitation and cleaning. During the session of the United Nations Environment Assembly in Nairobi, it was agreed that plastic pollution should be eradicated and a legally binding agreement should be created regarding this problem by 2024 (UNEP, 2022). To make this possible, more sustainable alternatives to conventional plastics (bio-based plastics and composite plastics) that have a low environmental impact should be considered (Spierling et al., 2018). The use of bio-based materials as an alternative to plastic promotes the formation of a circular economy and has numerous advantages such as recyclability/biodegradability, biocompatibility and low toxicity for the environment and humans (Teixeira-Costa and Andrade, 2021). Many countries that have recognized this global problem are taking significant steps towards suppression of the plastic pollution, such as limitation, reduced or even bans on the use of single-use plastics in at least 69 countries around the world (NEMA, 2017), increasing and improving the amount of plastic recycling by finding better technologies, more efficient and stricter waste and wastewater management, passing legal regulations in the field of plastic production and plastic waste management (Emmerik and Schwarz, 2019).

Companies are also making efforts to reduce their plastic impacts (Ellen Macarthur Foundation, 2017), while citizens are trying to break the habit of using single-use plastics by saying no to plastic bags, straws, bottles and other plastic products, replacing them with more sustainable and non-plastic ones. There are also efforts to clean rivers, lakes, seas and oceans through various cleaning actions. The legislation of the European Union has limited and banned the addition of plastics in cosmetic products and cleaning products, and stricter norms have been introduced for products such as textiles and tires in order to reduce the release of plastics from them (European Commission, 2019).

2. FROM PLASTIC TO MICROPLASTICS

2.1 Microplastics

According to the size, plastics are classified into: megaplastics (>100 mm), macroplastics (100-20 mm), mesoplastics (20-5 mm), microplastics (5-0.1 mm) and nanoplastics (<0.1 mm) (Barnes et al., 2009). The term microplastics (MP) is used to describe particles ranging in size from a few microns to a few millimeters (Bellasi et al., 2020). MP represents a heterogeneous group of particles that differ in size, shape, color, chemical composition, density and source (Sighicelli et al., 2018). Based on the source, MP can be divided into primary and secondary MP.

Primary MP represents particles that reach the environment in the range of size MP (0.1-5.0 mm) (Rios mendoza and Balcer, 2018). Primary MP is used in a wide range of industrial activities: 1) in the cosmetic industry (in creams, scrubs, baths creams, toothpastes, washing and whitening agents, etc.); 2) in medicine as vectors for drugs; 3) in air blasting technologies for removing rust and paint from surfaces; 4) as pre-production plastics (raw material for the production of plastic materials); 5) in the production of ink for 3D printers; 6) in the textile industry for fiber production (Browne et al., 2011; Rios mendoza and Balcer, 2018; Simon-Sánchez et al., 2019). The most important sources of primary MP in the environment are wastewaters through which MP is discharged from cosmetic products and washing machines (Carr et al., 2016; Murphy et al., 2016).

Secondary MP is created by the degradation (shredding) of larger plastic objects by physical, chemical and biological processes in the environment (Simon-Sánchez et al., 2019). Secondary MP makes the majority of MP accumulated in the environment (Peng et al., 2018).

2.2 Microplastics Impacts, Fate and Behavior in Aquatic Ecosystems

One of the most worrying forms of plastic pollution is the pollution of aquatic ecosystems by MP (UNEP, 2021), while one of the biggest ecological problems of MP pollution is that, unlike macroplastics, MP cannot be removed from the environmental matrix endangering life in aquatic ecosystems (Matjašič et al., 2022).

Most plastics/MP in the environment end up in aquatic ecosystems (Li et al., 2018). MP can reach aquatic ecosystems after direct discharge or can be transported from land. It is estimated that 80 % of waste in aquatic ecosystems is delivered from terrestrial sources: public waste, disposal of illegal waste, landfill runoff, tourism, industrial activity and combined sewage systems, while the remaining 20 % of waste to aquatic ecosystems is delivered from water sources (water traffic) (Bellasi et al., 2020).

MP in the aquatic environment can be transported and tie up persistent organic pollutants (POPs), heavy metals as well as invasive/pathogenic microorganisms (Lončarski, 2020). Variations in the MP density result in different behavior of synthetic polymer particles in the environment, more precisely the MP density depends on whether the MP will float on the surface of the water or settle on the sediment. MP tends to interact with components from the environment in which it is located, e.g. with humic/fulvic acid through aggregation/disaggregation and agglomeration/desagglomeration, and with an increase in the amount of MP in water, there is also an increase in its bioavailability to aquatic organisms, which affects its fate in aquatic ecosystems (Hidalgo-Ruz et al., 2012; Lončarski, 2020).

MPs can be ingested by a variety of aquatic organisms: zooplankton, crustaceans, fish, mammals and birds, which can lead to potential adverse effects on individuals, as well as their death (Murray and Cowie, 2011; Tanaka et al., 2013). MP poses a threat to biodiversity due to easy intake by aquatic organisms, and negative effects on living organisms can be physical and chemical.

A major challenge in assessing the chemical impact of MP on the environment is reflected in the presence of chemicals that can pose a danger to organisms. These chemicals include additives that are incorporated during production to give the plastic specific characteristics (Lithner et al., 2009; Teuten et al., 2009; Gallo et al., 2018; Besseling et al., 2019). Plastic materials have the ability to adsorb hydrophobic environmental pollutants and desorb them into habitats or into organisms (Lithner et al., 2009; Ogata et al., 2009). Adsorption mechanisms depend on factors, such as physicochemical properties of plastic/MP particles, properties of organic/inorganic compounds, as well as water chemistry (Ding et al., 2021). The presence of toxic chemicals in aquatic ecosystems increases the risks associated with the ingestion of MP by organisms, and many of the mentioned compounds are prone to biomagnification and can potentially pose a direct risk to human health as well (Rios et al., 2007).

In the case of physical effects, there are the size and shape of the MP particles. Physical side effects include: entanglement and swallowing of plastic objects (Webb et al., 2012). Aquatic organisms often become entangled in plastic remains, which can lead to serious injuries, limited ability to move, disability to feed and breathe properly (Gregory, 2009). Plastics/MP ingested by aquatic organisms and found in their digestive system can lead to reduced feeding stimuli, gastrointestinal blockage, reduced secretion of gastric enzymes, false satiety and decreased levels of steroid hormones that can also lead to reduced reproductive potential (Webb et al., 2012). Ingestion of plastics by aquatic organisms could increase human exposure through the food chain (Zhang et al., 2016; Emmerik and Schwarz, 2019). Apart from the aforementioned, MP can also serve as a refuge for different microbial communities or as a vector in the transmission of foreign (potentially pathogenic) microbial species (Lončarski, 2020).

The main ways of entering MP into the human body are ingestion (through food and water) and inhalation (through the respiratory tract) (Waring et al., 2018). MP entering the human body can cause a series of side effects and pose a potential risk to human health (Hwang et al., 2019). However, potential ecological risks as well as human health risks from MP are relatively new areas of research that need special attention.

Studies indicating MP ingestion by freshwater organisms are limited. Andrade et al. (2019) provide the first evidence of MP ingestion by freshwater fish of the Amazon, where about 80% of the analyzed species contained MP. The toxic effects of MP in freshwater ecosystems have not been sufficiently investigated (Bellasi et al., 2020). It is estimated that between 32 % and 100 % of freshwater organisms ingest MP (Watts et al., 2016). The possibility for organisms to ingest MP depends on the abundance and size of MP particles, the abundance of prey, and the physiological and behavioral characteristics of the organism. The size of particles that organisms can ingest depends on the physiology and morphology of the organism (Setälä et al., 2014).

2.3 Microplastics Origin in Aquatic Ecosystems

Plastics/MP arrive in aquatic ecosystems as a consequence of: industrial activities, insufficient and inadequate management of plastic waste, illegal disposal of waste, discharge of partially treated or untreated wastewater, unintentional or accidental release of plastics/MP into the environment (Crawford and Quinn, 2017; Simon-Sánchez et al., 2019).

The abundance of MP has been reported in various aquatic ecosystems: in seas and oceans (Browne et al., 2011; Cole et al., 2011; Ivar Do Sul and Costa, 2014; Cozar et al., 2014; Bergmann et al., 2017; Bošković et al., 2021, 2022a, 2022b, 2022c), in lakes (Eriksen et al., 2013; Free et al., 2014), rivers (Moore et al., 2011; Castañeda et al., 2014; Wagner et al., 2014) and estuaries (Sadri and Thompson, 2014; Wessel et al., 2016).

However, most studies report on the abundance, sources, concentration, transport routes, fate and impact of MP on biota in marine ecosystems as ultimate repositories of MP, while knowledge about the abundance, origin, distribution patterns, transport routes of MP on freshwater ecosystems is restricted (Moore et al., 2011; Carpenter et al., 2011; Eerkes-Medrano et al., 2015).

As freshwater ecosystems include rivers, lakes, streams, wetlands, ponds, etc. examination and pollution of freshwater ecosystems with MP is very complex because each of the water bodies it has different characteristics (hydrology, chemistry, flora and fauna). Freshwater ecosystems act as a receiver, sink and transporter of plastic pollution (Eerkes-Medrano et al., 2015; Horton and Dickon, 2018; Li et al. 2018; van Emmerik and Schwarz, 2020).

Interest in the study of MP in freshwater ecosystems has been increasing since 2013 (Eriksen et al., 2013). Examining the abundance of MP in freshwater ecosystems tends to close the gap in knowledge, occurrence, sources and fate of MP (Mani et al., 2016; Dris et al., 2018; Lahens et al., 2018; Campanale et al., 2019), and MP accumulation in freshwater ecosystems highlights the omniabundance of this form of pollution (Moore et al., 2011; Zbiszevski and Corcoran, 2011; Wagner et al., 2014; Scherer et al., 2017; Turner et al., 2019; Guerranti et al., 2020).

It is estimated that most part of the MP pollution of the marine ecosystems comes from terrestrial sources (80 %). MP that reaches the environment is partly transported to seas and oceans (through rivers), while the other part remains in freshwater systems (Lebreton et al., 2017; Yuan et al., 2019; Iannilli et al., 2020). Freshwater ecosystems, especially rivers, are considered one of the main sources of MP into the seas and oceans and the main transport vector of plastic waste from terrestrial sources (Iannilli et al., 2020), so the study of freshwater ecosystems is of great importance for identifying sources of pollution, dynamics, dispersion, accumulation and fate of MP in marine ecosystems as final depots of MP (Sighicelli et al., 2018; Dusaucy et al., 2021).

The sources of MP in freshwater ecosystems are numerous (Windsor et al., 2019; Simon-Sánchez et al., 2019), and the most important are considered to be: urban areas (wastewater from households, runoff from city and road surfaces, landfills and wild dumps), different industries and plastic production industries, atmospheric dust, fishing and agricultural activities (Matjašič et al., 2022).

Sources of MP pollution in urban areas are influenced by population density, wastewater treatment and waste management, traffic density, watershed area, industrial activity, and climate conditions (Ding et al., 2021). Waste generation in freshwater ecosystems can be consequence of waste disposal or accidental release during waste handling phases (Kum et al., 2005; Munoz-Cadena et al., 2012). Waste reaching freshwater ecosystems may already be in the MP size range or may act as a source of MP through the decomposition of larger plastics into fragments of micro size. Water transport is an important process for transporting plastic waste from urban land areas to nearby freshwater ecosystems (Ding et al., 2021). Impact of wind has also been identified as an important process of MP distribution from urban areas to freshwater ecosystems. Urban drainage systems represent the key path that connects urban and freshwater environments, so urban drainage is considered a channel through which MP reach freshwater ecosystems. Wastewater represents one of the most important sources of MP in freshwater ecosystems (Ding et al., 2021) which indicates that MP originates from households. Wastewater contains both primary and secondary MP (Rezania et al., 2018). Fibers account for 70 % of MP that is discharged from wastewater treatment plants (WWTP) (Emmerik and Schwarz, 2019). Brown et al. (2011) concluded that up to 1900 MP fibers can be released during a single cycle of machine wash.

Produced plastics are usually in granule or powder form. Plants for the production and processing of plastics, transshipment and transport can be significant emitters of MP that are "lost" during the processes and stages and are released into the environment. Also, plastic remains and raw materials that are kept and stored outdoors are exposed to wind and rain what can lead to their scattering and reaching the surrounding soil and water surfaces (Lončarski, 2020).

Road surfaces represent a complex anthropogenic environment characterized by mechanical abrasion from vehicle tires on the road surface (Sundt et al., 2014; Lassen et al. 2015; Magnusson et al., 2016). Road runoff is characterized by the abundance of particles with polymeric components, so road runoff can be considered as one of the significant sources of MP in the environment (Kole et al., 2017; Wagner et al., 2018; Vogelsang et al., 2019; Baensch-Baltruschat et al., 2020).

Atmospheric dust is not an insignificant source of MP (Dris et al., 2015). Atmospheric dust comes from urban areas, waste incineration, landfills, industrial emissions, traffic and use of fertilizers (Dris et al., 2018). Urban dust represents sinks but also sources of MP in aquatic ecosystems (Abasi et al., 2018). Intense precipitation (rain, storm and snow) and wind influence the increase of MP in aquatic ecosystems due to the washing of atmospheric dust as well as the remobilization of MP that temporarily accumulates in inshore zones (Dris et al., 2018; Dusaucy et al., 2021). Surface runoff can also be significant sources of MP depending on watershed characteristics and climatic conditions (Ding et al., 2021).

Research groups suggest that discarded fishing material such as fishing ropes and/or nets could be significant sources of MP in freshwater ecosystems. Also, a large amount of MP is released into aquatic ecosystems during fish farming (Deshpande et al., 2020). Based on the aforementioned, aquaculture activities are considered important sources of MP in aquatic ecosystems which could pose a threat to aquatic organisms and human health (Ding et al., 2021).

Agricultural activities represent another important anthropogenic activity that can be a source of MP in aquatic ecosystems. Agricultural soils are rich in plastic residues, and these materials can be transported in aquatic ecosystems (Nizzetto et al., 2016). Plastic culture is an important source of MP in agricultural soils. Agricultural soils have been identified as significant MP reservoirs, so it is predicted that agricultural soils may actually exceed the MP loads that are currently notices in the oceans (Nizzetto et al., 2016). Wind, surface runoff, flow and leaching are processes that can lead to the transmission of plastic particles from soil systems to freshwater ecosystems (Hurley and Nizzetto, 2018).

2.4 Microplastics in Freshwater Ecosystems

Freshwater ecosystems are in more direct and frequent contact with people (given that most of the world's population lives along rivers and lakes), compared to seas and oceans (Jiang et al., 2019). Freshwater ecosystems represent significant natural resources that support human life, economic development and are closely related to human wellbeing and aquatic organisms, so the study of MP in freshwater ecosystems is of great importance (Horton et al., 2017; Fahrenfeld et al., 2019). Freshwater ecosystems have many benefits for society based on ecosystem services such as: control services (floods and droughts), provision services (drinking water, food production through fisheries, aquaculture and irrigation of agricultural land, energy production through hydropower dams), cultural services (recreation), as well as favorable conditions for sustainable nutrient cycling (Dusaucy et al., 2021). Research on freshwater ecosystems is crucial for a better understanding of MP accumulation and sources in the oceans and global distribution patterns.

As there are still no legal regulations regarding the concentration of MP in water or developed technologies for their removal, there is an aspiration and need to find solutions for obtaining better quality of drinking water and "healthier" and cleaner freshwater ecosystems.

2.4.1 Microplastics in Rivers

Rivers play an important role in the transport of plastics/MP to lakes, seas and oceans (Bellasi et al., 2020). Rivers are considered the main transportation routes of plastics of various sizes, including MP. It is estimated that up to 2.75 million tons of plastic is transported by rivers into the oceans and seas every year (Lebreton et al., 2017; Schmidt et al., 2017). The characteristics of basin and the hydrogeomorphology of rivers affect the distribution and transport of MP. Heavy rainfall, wind, floods, level of water, erosion of soil can affect the distribution of MP particles within the catchment area (Bläsing and Amelung, 2018; Werbowski et al., 2021). Anthropogenic activities such as inadequate management of waste and wastewater, high population density near rivers can cause direct (or indirect) entering of MP into ecosystems of rivers (Schmidt et al., 2017). Flow conditions (speed of flow, flood events) and particle properties (shape, density, type of material) are the most important factors that control transportation of MP in ecosystems of rivers (Matjašič et al., 2022). Rivers can transport plastic waste over long distances to lakes, seas and oceans (Schirinzi et al., 2020).

Depending on the density of MP particles, MP can float or sink in ecosystems of rivers (Waldschlager and Schuttrumpf, 2019). MP particles of higher density sink into the riverbed (Nizzetto et al., 2016). Also, MP can accumulate and sink in river sediments when water flow becomes slower which results in increased concentrations of MP in river sediments (Horton and Deakon, 2018; Adomat and Grischek, 2020). Therefore, river sediment could act as a sink for deposition, retention and accumulation of MP, resulting in a hotspot of abundance of MP in the upper sediment layer (Hurley et al., 2018). Particles that settle in river sediment can be infiltrated into deeper sediment layers or resuspended again during conditions of stronger flow and currents of high-energy into the aqueous phase (Drummond et al., 2020).

The first data on the abundance of MP in rivers were published for European rivers: the Rhine (Klein et al., 2015), the Danube (Hohenblum et al., 2015), the Seine (Dris et al., 2015) and the Thames (Horton et al., 2017). Studies indicate that MP is present in different layers of the riverbed and water column (Guerranti et al., 2020). The ten largest Mediterranean tributaries, according to the annual flow, are the rivers: Rhone, Po, Bojana, Nile, Neretva, Ebro, Tiber, Adygea, Seyhan and Ceyhan. The Mediterranean rivers that contribute the most to plastic pollution in the Mediterranean are: the Adygea with 191 t of plastic waste per year, the Rhone with 208 t of plastic waste per year, the Neretva with 283 t of plastic waste per year, the Bojana with 575 t of plastic waste per year and the Po with 1349 t of plastic waste per year (Liubartseva et al., 2016).

2.4.2 Microplastics in Lakes

Lakes are considered closed or semi-closed systems with variable and different hydrographic conditions, and store water from precipitation, surface runoff, and groundwater (Sighicelli et al., 2018). Ecosystems of lakes are accumulators of pollutants, and therefore indicators of the basin's functioning. Lakes represent the final destination of MP transport compared to rivers, seas and oceans, which are subject to long-range and transport from several basins. In lakes, plastic debris remains are continuously accumulated and is deposited over a long period of time (Hidalgo-Ruz et al., 2012; Hardesty et al. 2017; Turner et al., 2019). The abundance, distribution and transport of MP in lakes is influenced by several factors: climatic variables (surface currents driven by the wind, storms, floods, runoff, water level), geomorphological characteristics (water depth, development of the area and coast), anthropogenic activities (lease of dams, tourism , fisheries) and the trophic chain (Fischer et al., 2016; Li et al., 2018; Alfonso et al., 2020).

MP was first recorded in the surroundings of the lake in 2012 (Faure et al. 2012). Lakes can have much higher MP concentrations than those recorded in oceans and seaside regions (Su et al., 2016) and represent major MP sinks in freshwater ecosystems. On the other hand, due to higher reserves of MP, lakes can also become an important source of MP for downstream watersheds (Yuan et al., 2019). Compared to rivers, lakes are characterized by slow water exchange, i.e. water stays in them longer (Kastratović, 2018). Due to the longer retention time in lakes, plastic remains can be decomposed in MP, settle or accumulate on shores, sediments and be available for uptake by aquatic organisms (Emmerik and Schwarz, 2019).

Located at the bottom of lakes and beneath layers of sediment, plastic particles are isolated from many degradation forces caused by weathering, like photodegradation (Corcoran et al. 2015). Studies that identified the presence of MP in sediment layers at a depth of up to 75 cm indicate that MP particles in the environment date from the beginning of the twentieth century, that is, from the beginning of plastic production (Turner et al., 2019). In lakes, sediments are the ultimate destination of transportation of MP. MP found in lake sediments are directly related to urbanization, industrial activities and impacts of wastewater (Corcoran et al., 2015; Driedger et al., 2015; Turner et al., 2019).

2.5 Sediments as Indicators of Microplastics Pollution

Plastics are very persistent, so it is estimated that it will take hundreds of years to degrade, and that the largest amount of MP will accumulate in sediments (Klein et al., 2015). Due to the absence or slow biodegradability, toxicity and harmfulness, long lifetime in the environment, entry into the food chain, MP is considered one of the most serious pollutants of aquatic ecosystems (Wang et al., 2016). Researches indicate that sediments are highly contaminated with MP particles (Hidalgo-Ruz et al. 2012; Vianello et al., 2013) and are considered the "final precipitator" of MP in aquatic ecosystems (Nizzetto et al., 2016).

The concentration of MP in sediments is significantly higher than the concentration of MP in the water column and that is why sediments are considered to be very good indicators for monitoring historical and current MP pollution (Peng et al., 2018; Adomat and Grischek, 2020). Sediments can reflect the long-term interaction between layers of water-sediment providing important information on the long-term accumulation, migration and fate of pollutants in aquatic ecosystems (Andrady, 2011; Wang et al., 2017; Peng et al., 2018).

Analysis of the quality of sediment is important for several reasons: quality of sediment indicates the current state of the aquatic ecosystem; sediment can be used to detect the abundance of pollutants that are not soluble in water and enable the assessment of pollution of aquatic ecosystems; analysis of sediment provides a precise picture of pollution over a long period of time; temporal trends of pollution can be analyzed based on the depth of the profile and levels of contamination can be estimated (Kastratović, 2018).

MP can move horizontally and vertically in aquatic ecosystems. Horizontal transport mechanisms are weaker and therefore less expressed, and are conditioned by the speed of flow, currents, precipitation, vegetation, but also the influence of the wind, through which MP can easily be transported and later accumulated on the banks of rivers and lakes (van Emmerik et al., 2018). Vertical transport of MP in the aquatic environment is more common and implies the movement of MP from the sediment surface to the water column and vice versa (Emmerik and Schwarz, 2019). MP can be resuspended from the sediment in the water column through hydrodynamics and interaction with organisms. Due to hydrodynamics during hydrological fluctuations, MP can float in the water column. Plastic density affects the vertical movement of MP (Li et al., 2018), so high-density MP will accumulate in sediments due to gravitational settling, while low-density MP can accumulate in sediment due to various physical, chemical, and biological factors (Corcoran, 2015; Li et al., 2019). Ingestion of MP by benthic organisms also affects the vertical movement of MP in aquatic ecosystems (Haegerbaeumer et al., 2019).

Sediments represent a habitat for many aquatic organisms, as well as a place where a large number of pollutants are deposited. MP that are accumulated/deposited in sediments can be available to benthic organisms that can mistakenly ingest it instead of food, increasing the toxicological risk to benthic fauna, disrupting the survival of individuals, affecting the trophic chain and the health of humans as the last link in the food chain (Murray and Cowie, 2011; Triebskorn et al., 2019).

Sediments have been identified as one of the major sinks of MP, while river-lake systems are the main transport routes of MP into the seas and oceans. Most studies focus on the abundance of MP in surface sediments (0-5 cm), because surface sediments are considered the most polluted layer (Wang et al., 2016). Inshore sediments represent the link between aquatic and terrestrial ecosystems, therefore they are particularly vulnerable and often show high MP concentrations (Hidalgo-Ruz et al., 2012; Rezania et al., 2018). In this way, assessment of MP contamination levels within inshore habitats is important for both proper risk analysis and assessment of plastic contamination trends (Piehl et al., 2019). Inshore sediments besides representing a very important zone of MP accumulation, collection of surface sediments on the banks of rivers and lakes offers a larger area for sampling, as well as a greater influence of natural (such as floods) and anthropogenic factors that can affect the quality and pollution that make the obtained results more competent and precise (Adomat and Grischek, 2020).

EXPERIMENTAL PART

3. MATERIALS AND METHODS

3.1 Area of Study

The nature of Montenegro is reflected in the beauty of its mountains, lakes, rivers and coasts. The rivers of Montenegro, in addition to their beauty, are characterized by a large amount of water during most of the year (Sekulić and Radulović, 2020). Montenegro has a specific hydrology, which is a consequence of the large distribution of karst (carbonate rocks such as limestone and dolomite). The territory of Montenegro is divided into two basins, the Black Sea and the Adriatic basin, Figure 3.1. The Black Sea basin covers 7.188 km^2 or 52 % of the territory, and it is consisted of the following rivers: Piva, Tara, Lim, Ibar and Ćehotina. On the other hand, the Adriatic basin covers 6.624 km^2 or 48 % of the territory, and the main rivers of the basin are: Morača, Zeta, Cijevna and Bojana (Barović et al., 2021). The largest part of the Adriatic basin belongs to the basin of Skadar lake. Skadar lake is drained by the Bojana river, which flows into the Adriatic Sea (Pešić et al., 2020; Sekulić and Radulović, 2020; Barović et al., 2021).

Figure 3.1 Division of Montenegro into two basins (Pešić et al., 2020)

Water can be chemically, physically and/or microbiologically contaminated, and water pollutants can be of inorganic, organic and/or biological origin (Kostić et al., 2016; Dorotan et al., 2018). As surface waters are particularly endangered, insight into water quality is equally important for both humans and aquatic organisms. In general, the quality of freshwater and seawater in Montenegro is continuously deteriorating. The lack of sewage network and the uncontrolled discharge of industrial and municipal wastewater contribute significantly to water pollution. As freshwater ecosystems (surface and underground waters) are used for water supply to the population, it is important that the quality of freshwater ecosystems is at a satisfactory level (Tomović, 2008).

In this dissertation, for the first time, surface inshore sediments of the rivers Zeta, Morača and Bojana, and Skadar lake were studied for the abundance of MP with the aim of ecological assessment and obtaining new knowledge.

3.1.1 Zeta

The Zeta river is located in the central region of Montenegro, flowing through Nikšić, Danilovgrad and Podgorica. Zeta originates in Nikšić field, more precisely in Gornje field from a large number of springs and rivers Sušica and Rastovac (Kračun-Kolarević et al., 2020). Zeta generally flows towards the south. It loses part of its waters through the existing sinkholes, then flows eastward to Glibavac, meandering towards the relief of the fields through the plain area of Mokra njiva. Part of its riverbed has been changed, so the Zeta flows south to Vukov bridge, and then through the concrete channels Zeta I and Zeta II to the compensation pool, where the waters enter the supply channel of HPP "Perućica". Zeta has an extremely high hydro potential (2.007 GVh/year) in the downstream zone of Nikšić (Đorđević et al., 2010; Sekulić, 2020). Before the construction of the hydropower system and the regulation of the riverbed of Zeta, the Zeta flowed through its natural abyss Slivlje in the Nikšić field as a sinkhole, in a length of about 5 km, and erupted again as a spring at the spring of Perućica and Glava Zeta. Up to Slivlje it is called Gornja Zeta, and from the spring Peručica it is called Donja Zeta. The Zeta flows through the Bjelopavlić plain mostly as a plain river with characteristic meanders. The Zeta receives several tributaries, permanent and occasional streams flow into it, and a large number of springs emerge along the riverbad of Zeta. It flows into the Morača river near Vranje fields. Zeta is the right and main tributary of Morača. The length of the Zeta river is about 89 km, and the catchment area is 1547 km² (Sekulić, 2020).

The lower course of the Zeta river with its surroundings has been declared a Nature Park. Due to the uncontrolled use of resources, the settlement of the population, the development of agricultural production, industrial facilities, illegal and uncontrolled waste disposal sites, and the spilling of wastewater, the flow of the Zeta is highly endangered. The disposal of various waste materials along the riverbed of the Zeta in its central and most upstream part is becoming more and more intense. Landfills in the immediate vicinity of the river bank, discharges of untreated wastewater that directly endanger living things, as well as surrounding settlements that negatively affect the quality of groundwater and soil have a particularly negative and harmful impact (Kračun-Kolarević et al., 2020).

3.1.2 Morača

The Morača is the longest Montenegrin river of the Adriatic basin, and at the same time the largest tributary of Skadar lake. Morača springs from under the mountain Rzace at an altitude of 975 meters, municipality of Kolašin. Morača flows through the municipalities: Kolašin, Podgorica and Cetinje, from north to south (Kračun-Kolarević et al., 2020). In the upper reaches of the Morača is a fast mountain river with torrential character and flows through the 38 km long Platije Canyon. The area of the Morača canyon is characterized by narrow and steep terrain slopes, interspersed with deep gorges, high mountains and mountain ridges. Morača receives several tributaries, the largest of which are Zeta (right tributary) and Cijevna (left tributary). Morača and its right tributary Zeta (the longest and richest tributary of Morača) are autochthonous rivers of Montenegro. The Cijevna river (left tributary) of Morače flows through Albania in a length of 23 km (Doderović et al., 2020). After merging with its largest tributary, the Zeta near Vranje filds (Podgorica municipality), the Morača enters the Zeta plain and flows all the way to the Vranjina hill in the Zeta plain, where it flows into Skadar lake. The Morača river is 113.4 km long, and the Morača basin covers an area of 2628 km² (Kračun-Kolarević et al., 2020, Kolarević et al., 2020; Pilua et al., 2022).

The Morača is considered one of the symbols of Podgorica and is the largest river that flows through this city. Morača and its most important tributaries, Zeta and Cijevna, flow through the most populated part of Montenegro, so they are under great pressure from various anthropogenic activities. The problem of pollution in the Morača river basin is most often a consequence of urbanization. Industrial activity in Montenegro has been significantly reduced due to the cessation of factories, so it can be argued that the share of industrial activities in the pollution of Morača has decreased (Doderović et al., 2020). Existing WWTPs partially purify water in the city area. The Environmental Protection Agency of Montenegro points out that communal wastewater is the biggest source of surface and groundwater pollution.

3.1.3 Bojana

The Bojana river originates from Skadar lake, near the city of Shkodër in Albania, so its flow depends on the water level in Skadar lake. The largest part of Bojana is the border river between Montenegro and Albania. The length of the Bojana watercourse is about 41 km, of which about 17.5 km belongs to Albania (Barović et al., 2021). On the territory of Albania, the rivers Kiri and Drim flow into Bojan, which significantly affect its flow. The Bojana is a navigable international river along its entire course for smaller cargo and passenger ships (Pantelić et al., 2020; Barović et al., 2021). It flows into the Adriatic Sea, it is the largest tributary of the Adriatic Sea in Montenegro, the second most important tributary of the Adriatic Sea after the Po river and the third river in terms of the amount of water that flows into the Mediterranean Sea after the Nile and Po rivers (Petković and Sekulić, 2018; Barović et al., 2021). Bojana meanders, and at the confluence with the Adriatic Sea near the village of Sveti Nikola, long-term deposition of sediment forms a delta, a triangular river island called Ada Bojana. Ada Bojana is located in the extreme southeast of Montenegro, along the state border with Albania. The island of Ada Bojana divides the Bojana river into two parts that flow into the Adriatic Sea as separate watercourses (Barović et al., 2021). The right watercourse of Bojana belongs to Montenegro, and the left one represents the international border between Montenegro and Albania. The bottom of the Bojana riverbed, from the mouth, is below sea level for a length of 36 km upstream. In the riverbed of the river Bojana there is sea salt and fresh river water. Seawater, as a heavier fraction, penetrates the bottom of the Bojana river bed about 8 km upstream from the mouth, to Sveti Đorđe (Petković and Sekulić, 2018). Due to all its characteristics and specificities, the Bojana river represents a natural phenomenon, a unique ecosystem in Europe and a natural reserve of flora and fauna (Pantelić et al., 2020). Delta of the Bojana river was declared a protected area, ranking among the most important wetlands in the Eastern Mediterranean (Petković and Sekulić, 2018).

Ada Bojana is the most famous touristic area in Montenegro. The pollution of Bojana is an international problem, it can be polluted from several directions: Albania, Montenegro, as well as from Kosovo and Macedonia by the Drim river, one branch of which flows into Bojana (Barović et al., 2021).). The Bojana flows out of Skadar lake, so the water quality of Bojana depends on the level of water pollution in Skadar lake. Wastewater from Nikšić, Danilovgrad and Podgorica is discharged into Skadar lake, and through Rijeka Crnojevića, wastewater from Cetinje can also reach it, which significantly affects the quality and pollution of Bojana. Also, it is important to mention that Bojana is threatened by wastewater and solid waste from Shkodër. Numerous cottages and restaurants were built along the course of the Bojana, the wastewater of which flows directly into the river (Barović et al., 2021). Large amounts of pollutants reach the Adriatic Sea via Skadar lake and the Bojana river (Pantelić et al., 2020).

3.1.4 Skadar Lake

Skadar lake is the largest lake on the Balkan Peninsula shared by Montenegro and Albania. It is located in the lower parts of the Zeta-Skadar plain (depression), which is partly a cryptodepression, which means that in some parts the bottom of the lake is below sea level, such places are also called eyes (Vemić et al., 2014). Approximately 65 % of the surface of Skadar lake belongs to Montenegro, and 35 % to Albania. The length of Skadar lake is about 44 km, the width is about 14 km, while the surface varies between 353 and 500 km^2, which depends on the seasonal nature of precipitation/evaporation, low water levels in summer and large floods during the winter months (Pešić et al., 2020). The basin of Skadar lake covers 5631 km^2, of which about 81 % belongs to the territory of Montenegro. Skadar lake has several tributaries from which it receives water, and the most important river that contributes 63 % of the total water inflow to the lake is Morača (Barović et al., 2018). The remaining water inputs to the lake are precipitation, wells and other smaller watercourses (Vemić et al., 2014). Skadar lake belongs to the flowing type of lake, so its water is released via the Bojana river in Albania, which flows into the Adriatic Sea in Montenegro. The lake basin belongs to the Adriatic basin (Vemić et al., 2014). Due to the specific hydrological phenomenon and physical-geographic characteristics of the lake, the water in the lake changes completely up to three times a year (Barović et al., 2018).

Skadar lake is an area of regional importance, it has an exceptional richness of ornithofauna and ichthyofauna as well as lush vegetation, it is also characterized by a wealth of cultural and historical monuments, islands and beautiful untouched nature. The lake is known for a wide range of endemic and rare, or even endangered plant and animal species (Vemić et al., 2014). Skadar lake was: declared a National Park in 1983, when it was protected; internationally recognized important bird area (IBA-Important Bird Area) in 1989; included in the Ramsar list of wetlands of international importance in 1996; and the Berne Convention Committee nominated Skadar lake in 2011 as an EMERALD area (Barović et al., 2018; Krivokapić, 2021).

Skadar lake is under big influence of anthropogenic activities that take place in its surroundings (Kastratović, et al., 2016; Pešić et al., 2020). The quality, protection and pollution of the lake are equally affected by anthropogenic activities carried out in Montenegro and Albania. Unwanted material from illegal landfills also arrives in Skadarlake, through numerous rivers and streams (Barović et al., 2018). The Center for Ecotoxicological Research of Montenegro (CETI) has identified the main factors that represent threats to the environment of Skadar lake and its basin: (1) industrial pollution (solid and liquid waste); (2) household pollution (municipal waste, wastewater); (3) fishing activities; (4) development and infrastructure on the shores of the lake; (5) increased expansion of tourism; (6) the impact of the agricultural sector; (7) problems caused by floods (Kostianoy et al., 2018).

3.2 Sediment Sampling

Sampling of the surface inshore sediment of rivers and lake was carried out in two periodic cycles, spring and autumn, more precisely, sampling began in the autumn of 2022 and ended in the spring of 2023.

On the examined water bodies, 4 to 6 sampling locations were selected. The locations included in the research were chosen based on the specific characteristics of the terrain, different geographical location, the possibility of access to them, as well as various anthropogenic activities in their immediate vicinity. Table 3.1 shows the locations of sampling, coordinates in relation to them and periodicity of sediment sampling. Figure 3.2 shows the entire study area with sampling locations, and Figures 3.3–3.6 show separately the studied water bodies, rivers: Zeta, Morače, Bojana and Skadar lake.

Table 3.1 Sampling locations, coordinates in relation to the same and periodicity of sediment sampling

Water body		Locations	Coordinates	Sampling seasons	
				Autumn 2022	*Spring 2023*
Zeta	Z1	Near the source of the Zeta	42.823724 18.914579	28.10.2022.	23.04.2023.
	Z2	Center of Nikšić	42.780583 18.922085	28.10.2022.	23.04.2023.
	Z3	Near the spring after sinking	42.667863 18.996957	28.10.2022.	23.04.2023.
	Z4	Center of Danilovgrad	42.554142 19.104908	28.10.2022.	23.04.2023.
	Z5	Zeta flows into Morača	42.468728 19.257833	28.10.2022.	23.04.2023.
Morača	M1	Near the source of the Moraˇca	42.487864 19.309921	28.10.2022.	23.04.2023.
	M2	Center of Podgorica I	42.447686 19.259313	28.10.2022.	23.04.2023.
	M3	Center of Podgorica I	42.439137 19.258194	28.10.2022.	23.04.2023.
	M4	Near the wastewater collector	42.434104 19.231581	28.10.2022.	23.04.2023.
	M5	Morača flows into Skadar lake	42.333727 19.209857	28.10.2022.	23.04.2023.
Bojana	B1	Border MNE-Al	41.954473 19.350085	29.10.2022.	07.05.2023.
	B2	Before the river island	41.879154 19.375965	29.10.2022.	07.05.2023.
	B3	Ada Bojana – right watercourse	41.870862 19.352894	29.10.2022.	07.05.2023.
	B4	Bojana outflow into Adriatic Sea	41.866275 19.339421	29.10.2022.	07.05.2023.
Skadar lake	S1	Rijeka Crnojevića	42.354681 19.028071	29.10.2022.	07.05.2023.
	S2	Karuč	42.356831 19.106510	29.10.2022.	07.05.2023.
	S3	Vranjina	42.272047 19.122810	29.10.2022.	07.05.2023.
	S4	Central part	42.267300 19.112934	29.10.2022.	07.05.2023.
	S5	Virpazar	42.246137 19.092805	29.10.2022.	07.05.2023.
	S6	Donji Murići	42.163509 19.221570	29.10.2022.	07.05.2023.

The locations chosen for sampling the inshore surface sediment of the rivers can be divided into three groups: (1) the source, that is, near the source of the rivers, locations that are considered the cleanest (Z1, M1); (2) the middle of the river course, locations exposed to the greatest anthropogenic influences (Z2-Z4, M2-M4, B1-B3) and (3) the mouth, the inflow of rivers, locations that indicate the pollution that the said river carries with it and therefore affects pollution of another river, lake or sea (Z5, M5, B4), Figures 3.3–3.5. The sample of the Bojana river near its source was not taken because the Bojana does not originate in the territory of Montenegro, so the first sample of the inshore surface sediment of the Bojana river was taken at the location where the Bojana enters the territory of Montenegro. The locations chosen for sampling the inshore surface sediment of the lake covered all sides of Skadar lake, with varying degrees of anthropogenic influence, and which belong to the territory of Montenegro, Figure 3.6.

Sampling of the surface inshore sediment of rivers and lake, at a depth of up to 5 cm, at each location was performed linearly at the water splash line using a stainless steel spoon/shovel, in accordance with the proposed methods available in the literature (Hidalgo-Ruz et al., 2012; Abidli et al., 2017; Yang et al., 2020). Möller et al. (2020) and Adomat and Grischek, (2020) state that the upper 0-5 cm of surface inshore sediment contains the highest proportion of MP in the sediment.

During sampling, three sediment samples were taken from one location and then homogenized (composite sample of three samples from one location), resulting in one sample of about 2 kg of wet sediment from each sampling location. Recommendations for the mentioned sampling method were proposed by Klein et al. (2015) and Jiang et al. (2018). Composite sediment samples were stored in labeled glass jars and then transported to the laboratory for further processing and analysis. Wet samples were sieved through metal sieves with a mesh size of 5 mm to separate pieces of macroplastics and larger sediment granulometry, while sediment samples $<$ 5 mm were subjected to a drying process.

Figure 3.2 Study area with sampling locations

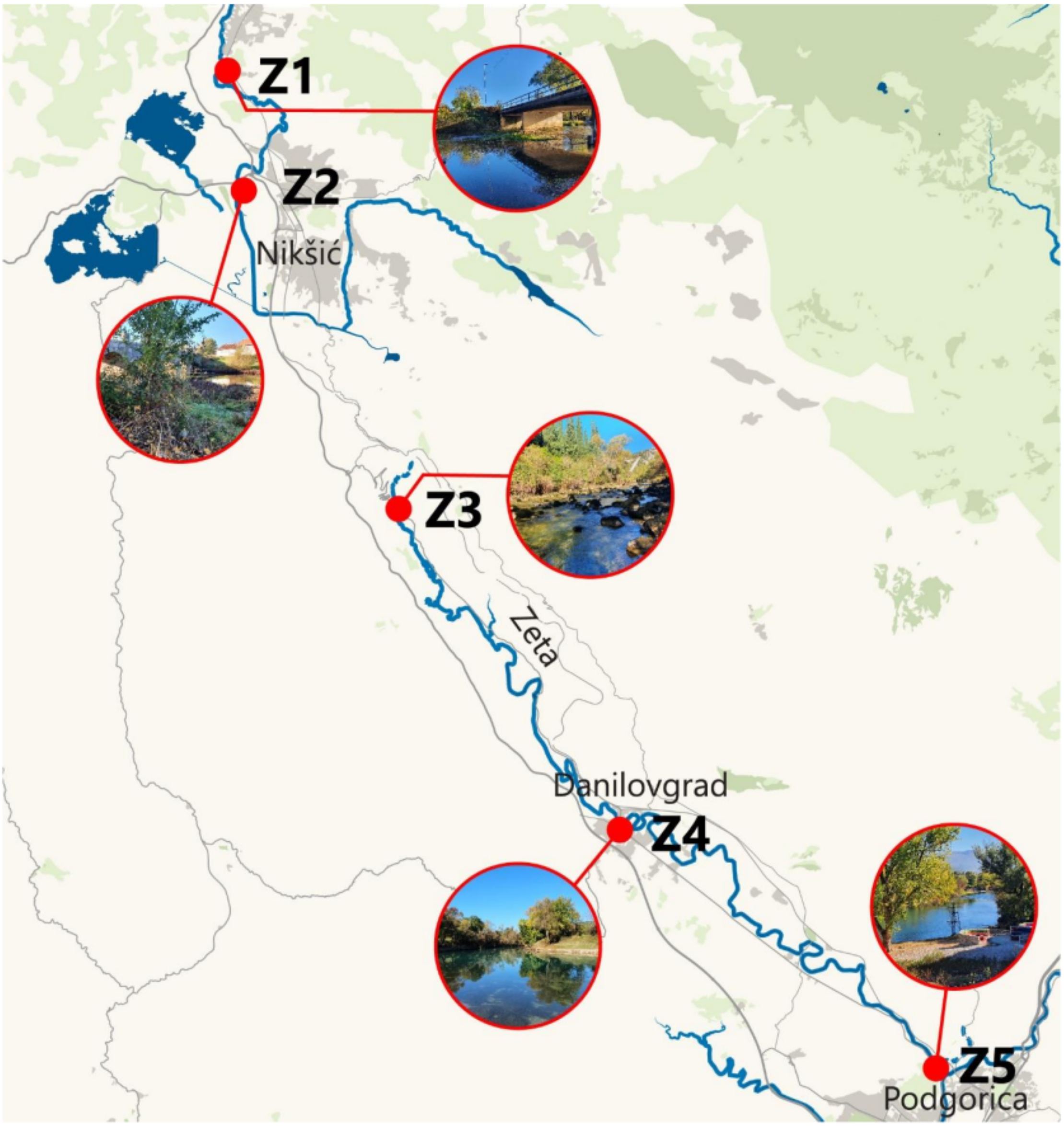
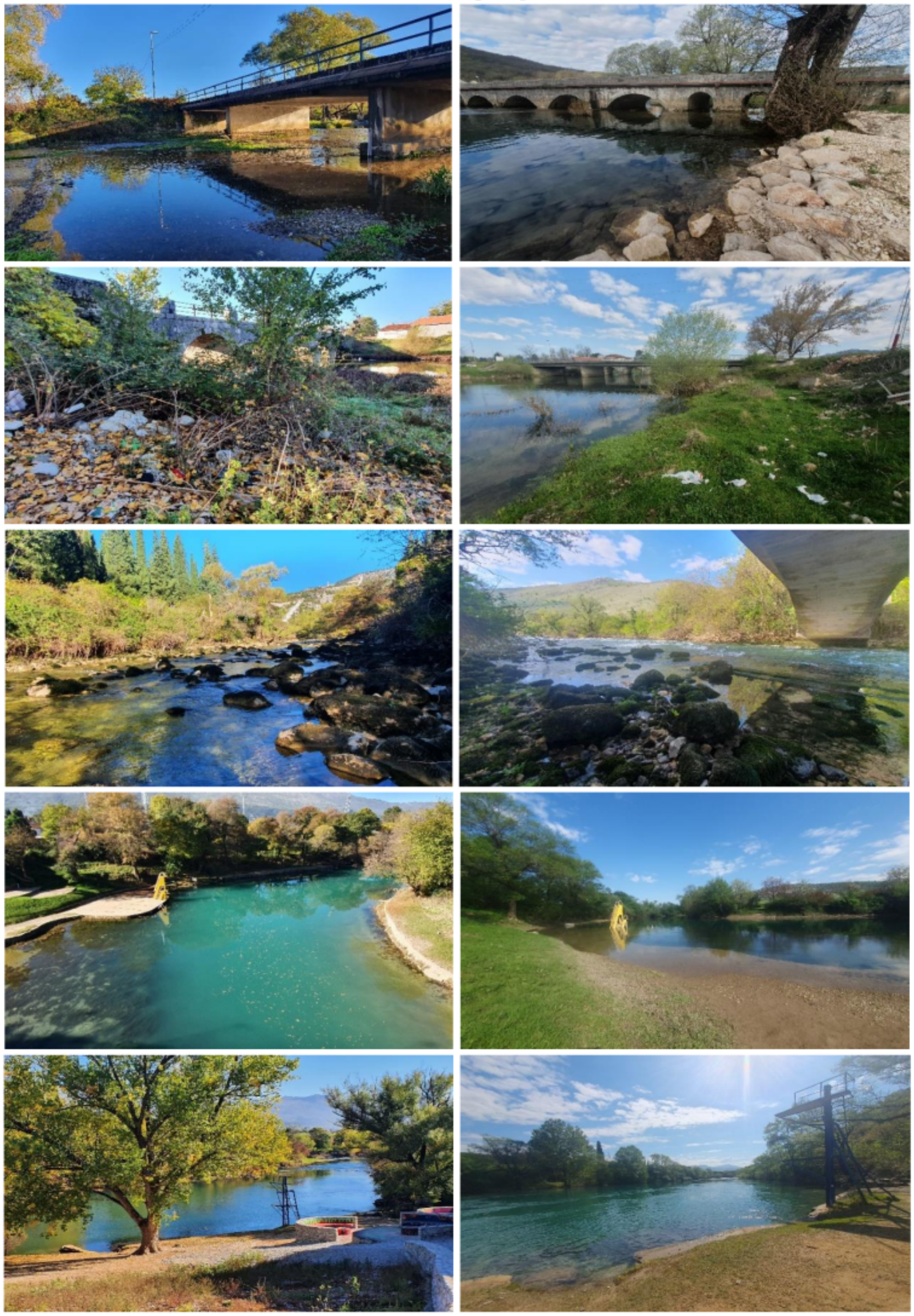


Figure 3.3 Locations of sediment sampling on the Zeta river

Autumn 2022 Spring 2023



Z1
Z2
Z3
Z4
Z5

Figure 3.4 Sampling locations on the Zeta river in relation to the sampling season

Figure 3.5 Locations of sediment sampling on the Morača river

Autumn 2022 Spring 2023

M1
M2
M3
M4
M5

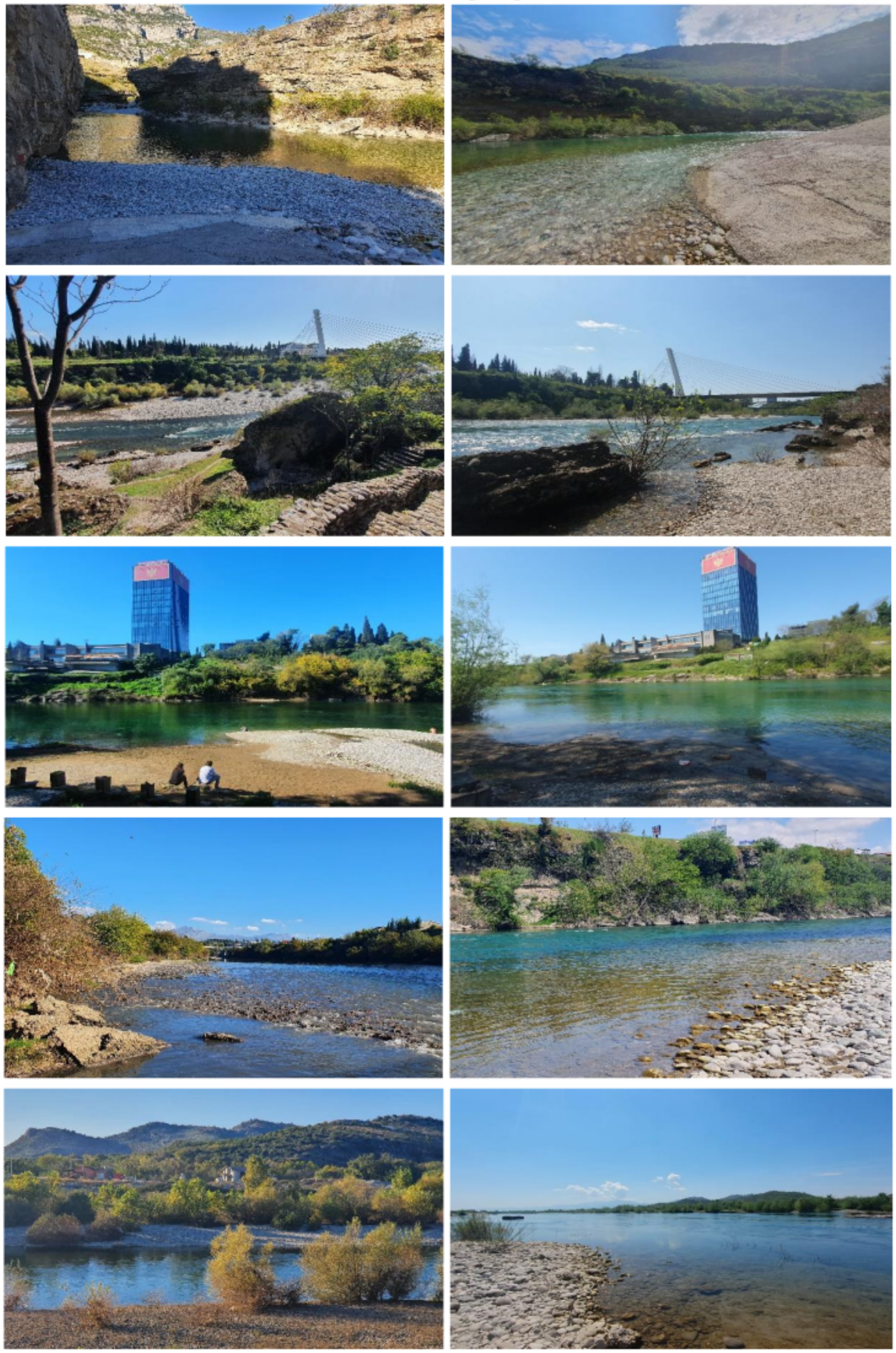


Figure 3.6 Sampling locations on the Morača river in relation to the sampling season

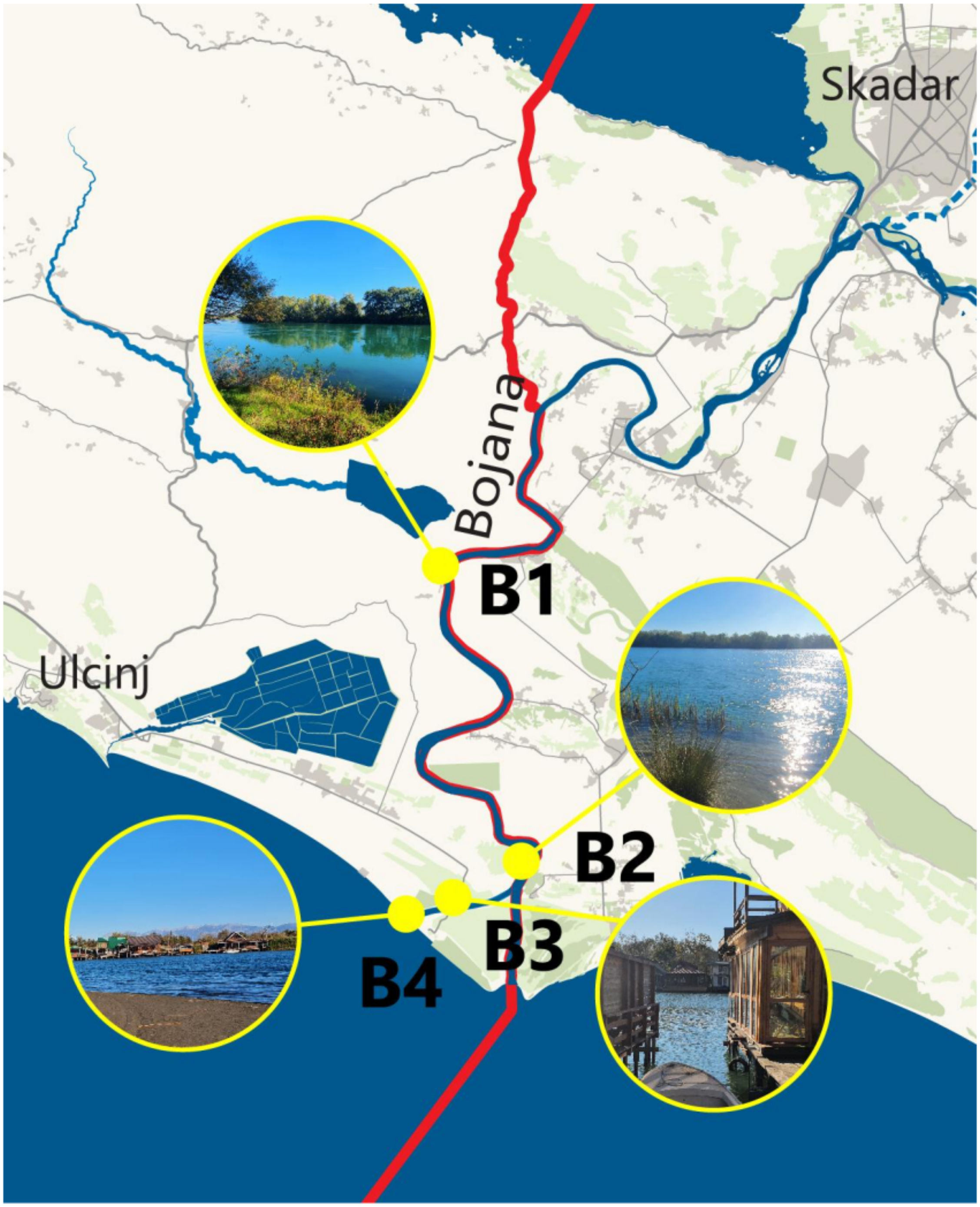


Figure 3.7 Locations of sediment sampling on the Bojana river

Autumn 2022 Spring 2023

B1

B2

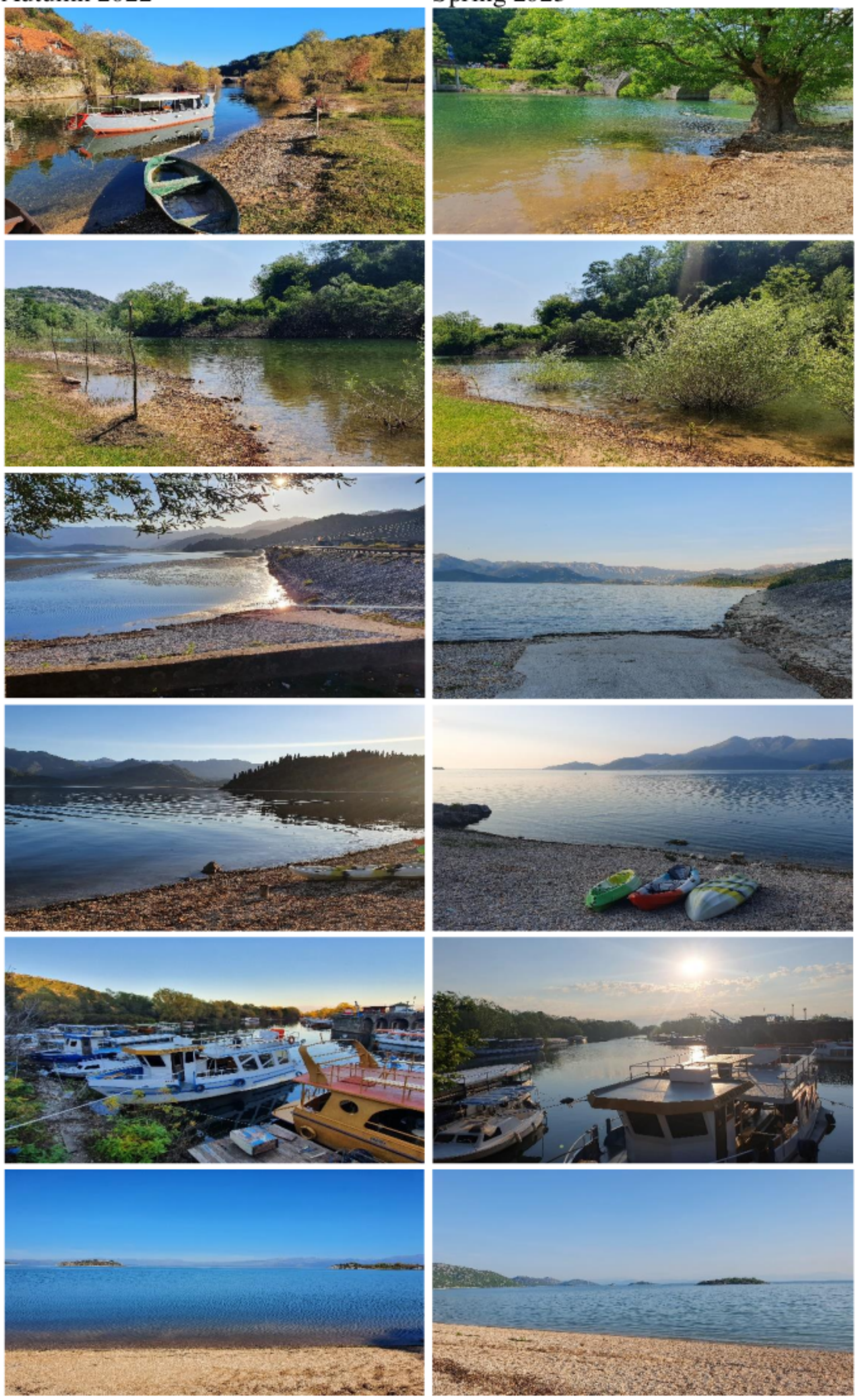
B3

B4

Figure 3.8 Sampling locations on the Bojana river in relation to the sampling season

Figure 3.9 Locations of sediment sampling on the Skadar lake

Autumn 2022 Spring 2023



S1 S2 S3 S4 S5 S6

Figure 3.10 Sampling locations on the Skadar lake in relation to the sampling season

3.3 Extraction of Microplastics from Samples of Sediment

Procedures for the preparation and extraction of samples of sediment for analysis of MP were carried out in the laboratories of the Faculty of Metallurgy and Technology of the University of Montenegro in Podgorica. The procedures for extracting MP from samples of sediment include the following stages: drying, separation of density, sieving, filtration and, if necessary, decomposition of organic matter.

3.3.1 Drying

Given that the mass of sediments varies and largely depends on the water content and type of sediments, it is recommended to use dried sediment to determine the MP content, which will provide more reliable results and increase comparability with literature data from the region and the world (Yang et al., 2020).

Samples of the wet sediment, placed in aluminum containers, were subjected to the drying process in the DRYSCN43 dryer that ensures a uniform temperature (Figure 3.11). Sediment samples were dried for 48 h at a temperature of 36 °C. Samples of the dried sediment were stored in glass jars. Figure 3.12 shows the process of drying of the sediment.

Figure 3.11 DRYSCN43 dryer with vertical hot air circulation

Figure 3.12 Sediment drying procedure

3.3.2 Separation of Density

Separation of density is a technique applied to extract MP from the samples of sediment, and is based on the difference in densities between the material of interest (MP) and other not plastic components (Hidalgo-Ruz et al., 2012). The specific density of plastic particles (range from 0.8 to 1.4 g/cm^3) can vary depending on the type of polymer and the process of production. In order to separate the plastic (lighter) from the non-plastic components (heavier) in the sediment sample, the selected saturated solution should be mixed with the sediment sample for a certain time, which will separate the plastic components based on the difference in density. Heavier sediment particles (high density) will settle to the bottom after settling, while lighter plastic particles (low density) will be separated in suspension, i.e. in the aqueous phase of the solution. As recommended by Thompson et al. (2004), saturated solution of sodium chloride - NaCl (concentration 5.475 mol/L, density 1.202 g/cm^3, solubility 360 g in 1 L of water), was used as an agent for separation of densities. The advantages of using the saturated solution of NaCl for separation of density in procedures of extraction of MP from samples of sediment are: the solution is not toxic, it is recyclable, economic, easy to handle and efficiency increases when repeating the procedures, possibility of comparison with previous researches, etc. (Van Cauwenberghe et al., 2015; Hanvey et al., 2017).

Using a saturated NaCl solution, MP particles with a lower density were separated in the water phase, while particles like sand grains, with a higher density, settled to the bottom. In order to increase the efficiency of extraction of MP from sediment samples (Nuelle et al., 2014), the procedure of the separation of density was performed three times with recycled saturated NaCl salt solution. Figure 3.13 shows the procedure of the separation of density.

Figure 3.13 Density separation

In glass jars with a volume of 1 L, 100 g of dry sediment sample was measured by the quartering process and 500 ml of saturated NaCl solution was added. The suspension was vigorously shaken for 5 minutes and then left for 48 h at room temperature to separate fractions based on density. The supernatant was decanted through a steel sieve, and the rest of the precipitate, for each sample, was subjected to the density separation process two more times.

3.3.3 Sieving and Filtration

Sieving is a technique for extraction of MP from the samples of sediment that is used after the process of separation of density. MP can be separated from the samples using a sieve of a certain mesh size. Materials retained on the sieve are kept for further analysis, while material passing through the sieve is discarded. Sieving is applied before the final extraction step to reduce the sample volume for the subsequent MP identification method, as well as to remove particles such as clay and silt. The supernatant is decanted through a sieve that physically retains particles of the desired dimensions and enables the removal of water from the sample. A stainless steel sieve with a mesh size of 63 μm was used for sieving, Figure 3.14. The content remaining on the sieve was quantitatively transferred into a glass beaker, and as recommended by Zobkov and Esiukova (2018), the sieve was thoroughly washed with deionized water after each sieving to minimize MP loss on the sieve walls.

Figure 3.14 Retsch test sieve: Ø 200 mm, height 50 mm, mesh size 63 μm

Sieving is followed by filtration with the application of a vacuum pump in order to separate MP from the liquid. Glass filters (GF/C Glass Microfiber Filters, 1.2 μm, 4.7 cm, Whatman 1822-047) were used for the filtration process. As recommended by Zobkov and Esiukova (2018), the walls of the laboratory filtration equipment were washed with deionized water several times during the filtration process to retain all the MP on the filter and prevent its loss. Filter papers were transferred with tweezers to glass Petri dishes where they were left to dry at room temperature. Figure 3.15 shows used filters and apparatus for the filtration process.

Figure 3.15 Filters and apparatus for the filtration process using a vacuum pump

3.4 Identification of Microplastics

MP identification consists of visual and chemical identification. Visual identification was performed in the laboratories of the Faculty of Metallurgy and Technology of the University of Montenegro in Podgorica, while chemical identification of MP in sediment samples was performed in the laboratories of the Marine Biological Station of the National Institute of Biology in Slovenia in Piran.

3.4.1 Microplastics Visual Identification

Visual identification of MP particles is often used in methodological approaches for initial enumeration and identification (Hidalgo-Ruz et al., 2012; Bošković, 2022a), and is performed by observation with the help of an optical microscope (Van Cauwenberghe et al., 2013). It is necessary to identify MP visually carefully and separate it from other non-plastic materials from the sample, such as organic remains (biogenic plant and animal remains) and other objects (stones, glass, paper, minerals, metals, etc.) (Hidalgo-Ruz et al., 2012). The dried filters were visually inspected under a STEBD optical professional microscope (Figure 3.16) to determine the abundance of potential MP particles. The entire surface of the filter was examined, and the MP particles on the filters were counted three times, in order to reduce the counting error. Visual identification of MP is based on physical and morphological characteristics of particles, such as shape, size and color (Yang et al., 2020; Bošković et al., 2022c). To avoid misidentification and underestimation of MP and to guarantee the correct identification of MP particles, the criteria proposed by Norén (2007) were followed: (1) MP particles did not possess cellular structures; (2) fibers were of consistent thickness and color throughout their length; (3) clear and white MP particles were confirmed under a high-magnification microscope.

MP is classified according to the shape type into four categories: fragments, films, fibers and granules (Zhang et al., 2017; Zobkov et al., 2020). Fragments are pieces of thicker plastic, irregularly shaped, rigid, with sharp curved edges. Films are thin sheets of plastic bags and other similar items, irregularly shaped, thin, flexible and usually clear compared to fragments. Fibers are thin elongated particles or threads, one dimension of which is significantly larger than the other two, they can look like ribbons or they can be cylindrical in shape. Granules are three-dimensional particles, spherical, like resin pellets (Claessens et al. 2011; Frias and Nash, 2019; Jiang et al., 2019), Figure 3.17.

Figure 3.16 STEBD optical professional microscope

The size of MP particles is divided into four size categories: 0.1–0.5 mm; 0.5–1 mm; 1–3 mm and 3–5 mm (Dusaucy et al., 2021).

The color classification of MP particles was carried out according to the following categories: clear, white, gray, brown, green, yellow, pink, red, blue and black, whereby dark blue, light blue and purple are included in the blue category, cyclamen in the pink category colors, and orange in the red color category (Zobkov et al., 2020). Although the color of MP particles is not considered a key category for defining MP, as color determination is subjective, the categorization of MP according to color is useful for identifying potential sources of MP as well as potential contamination (Hartmann et al., 2019; Bošković et al., 2022b).

Figure 3.17 Shape types of microplastics

Visually identified MP particles were quantified for each sample individually, photographed and saved, as well as classified according to shape, size and color categories. Each visually identified MP particle was photographed using a microscope and software. The abundance of MP particles in sediment samples was expressed as the number of MP particles in 100 g of dry sediment, which was converted into the number of MP particles per kg of dry sediment, while the abundance of different types of shape, size and color of MP was expressed as a percentage abundance.

3.4.2 Microplastics Chemical Identification

Fourier-transform infrared spectroscopy (FTIR) is a reliable and accurate instrument and analytical method for the identification of MP polymer type (Browne et al., 2010; Hidalgo-Ruz et al., 2012; Lusher et al., 2014; Rezania et al., 2018). FTIR spectroscopy uses modulated, mid-infrared (IR) energy to examine the sample, i.e. the structure of individual molecules and the composition of molecular mixtures. IR light is absorbed at specific frequencies that are directly related to the energy of the atom-atom vibrational bond in the molecule. When the energy of the bond vibration and the energy of the mid-IR light are equivalent, the bond can absorb that energy. The position (frequency) and intensity of individual absorption bands contribute to the overall spectrum, creating a characteristic imprint. IR radiation interacting with MP particles leads to the creation of characteristic patterns of IR absorption (spectra). Polymer identification is obtained by comparing known reference FTIR spectra with the obtained spectrum (Simon-Sánchez et al., 2019; Yang et al., 2020). Attenuated Total Reflectance (ATR) and μFTIR spectroscopy were used in the dissertation. ATR-FTIR is widely used in the identification of larger MP particles (Ivleva et al., 2017), and the advantages are based on providing a strong particle-to-crystal ratio and obtaining a clearer spectrum. For smaller particles, μFTIR spectroscopy was applied, which combines FTIR spectroscopy with microscopy in the IR ranges (Yang et al., 2020). The detection of MP is based on the stimulation of the molecular vibration with IR radiation and the molecular structure of the substance and the wavelength, i.e. the contact of the particle with the crystal during which the IR rays pass through the crystal, come into contact with the particle and are reflected back forming a spectral imprint (Hidalgo-Ruz et al., 2012; Bošković, 2022a).

Plastic polymers have specific IR spectra, which enables FTIR to accurately identify polymer particles according to their characteristic IR spectrum (Löder and Gerdts, 2015; Bošković, 2022a). The generated spectra are compared to a database of known polymers and other materials to confirm the exact identity of the compound. As the matching of the obtained spectra with the known spectra from the database varies from 1-100 %, results with a match of more than 70 % were considered positive, and the particles were considered MP.

In each sediment sample, a minimum of 30 % of visually identified MP particles with different morphological and physical characteristics (different types of shapes, color categories and sizes) were chemically identified in order to correct potential overestimations of MP by visual identification due to the counting of non-plastic components such as cellulose, inorganic and organic matter, as well as the identification of the types of polymers present in the sediment samples.

The results of the chemical identification of polymer types were corrected according to the level of identified non-plastic components for each sample individually to eliminate overestimation of the results. The results are expressed as a percentage for each sediment sample. Perkin Elmer FTIR microscope Spotlight 200i with ATR FTIR Spectrum Two (Figure 3.18) is the instrument used for chemical identification of MP. The number of scans per particle was 16, with the final spectrum being the average of all 16 scans. The frequency range of FTIR is set in the range from 4000 cm^{-1} to 500 cm^{-1}.

Figure 3.18 Perkin Elmer microscope Spotlight 200i with ATR FTIR Spectrum Two

3.5 Ecological Assessment of Risk of Microplastics

Potential risks to the environment and human health from the identified polymers in the examined sediment samples were checked by the European Chemical Agency - ECHA (www.echa.europa.eu/home). Impacts on the environment and human health are based on Harmonized classification and labeling - CLH, which is harmonized with the European Commission EC No 1907/2006 of the European Parliament and the Council of the Registration, Evaluation, Authorization and Restriction of Chemicals - REACH.

Standard and systematic model for assessing the potential ecological risks of contamination of MP does not exists (Wang et al., 2021). Based on the above, many scientists have developed a risk assessment model that is applicable to other pollutants as well, in order to better understand and assess the environmental risks caused by MP pollution. The ecological risk assessment of MP pollution in the Zeta, Morača and Bojana rivers and Skadar lake was carried out based on previous studies (Lithner et al., 2011; Xu et al., 2018; Peng et al., 2018; Pan et al., 2020 ; Wang et al., 2021;

Ranjani et al., 2021; Gurumoorthi and Lewis, 2023). In this regard, in this study, Pollution load index - PLI and Polymer hazard index - PHI were applied to assess the potential ecological risks of MP.

To assess the degree of MP pollution in river and lake sediments, the integrated PLI proposed by Tomlinson et al. (1980) was calculated. The classification criteria for the level of risk are presented in Table 3.2. PLI is calculated according to the following formulas:

$$CF_i = C_i/C_{0i} \quad (1)$$

$$PLI_i = \sqrt{CF_i} \quad (2)$$

$$PLI_u = \sqrt[n]{PLI_1 \times PLI_2 \times \cdots \times PLI_n} \quad (3)$$

where: CF_i is the MP concentration factor, which represents the quotient of the MP concentration at each location and the background concentration; C_i is the MP concentration at location *i*; C_{oi} is the background concentration of MP. The lowest MP concentration detected in river and lake sediment samples was considered as background value (Wang et al., 2021; Ranjani et al., 2021; Gurumoorthi and Lewis, 2023). PLI_i is the MP pollution load index at location *i*; PLI_u is the MP pollution load index in the river/lake during the entire study, and *n* is the total number of investigated locations in the river/lake.

The chemical toxicity of different types of MP polymers identified in river and lake sediments was calculated based on MP concentrations in river and lake sediments and the chemical composition of each type of plastic polymer taking into account the hazard rating obtained by Lithner et al. (2011). Lithner et al. (2011) categorized the chemical toxicity of different types of polymers into five categories, Table 3.2. PHI is calculated using the following formulas:

$$H_n = P_n \times S_n \quad (4)$$

$$PHI = \sum H_1 + H_2 + H_3 \ldots H_n \quad (5)$$

where: H_n is the polymer hazard index; P_n is the percentage of specific *n* types of polymers identified in river and lake sediments; S_n is the hazard rating of each type of MP polymer identified in river and lake sediment, derived from Lithner et al. (2011), Table 3.2; PHI is an index of the polymer hazard caused by all identified polymers in rivers and lake.

Table 3.2 Used terminology of hazard level criteria for environmental MP risk assessment

PLI	Hazard category	PHI	Hazard category	Hazard score for polymers (Lithner et al., 2011)	
<10	I	0-1	I	Polytetrafluoroethylene (PTFE)	x
10–20	II	1-10	II	Polyvinyl alcohol (PVA)	1
20–30	III	10-100	III	Polypropylene (PP)	1
>30	IV	100-1000	IV	Polyethylene terephthalate (PET)	4
		1000-10000	V	Polyvinyl chloride (PVC)	10.5
				Polyethylene (PE)	11
				Polystyrene (PS)	30
				Polyamide (PA)	47
				Acrylate copolymer (Acriate cop.)	1021

3.6 Quality Assurance and Control

Procedures of quality control are important when analyzing MP in environmental samples. Overestimation (false positive results) can occur as a result of background contamination of the sample, while underestimation appears as a loss of analytical material during analysis (Zobkov et al., 2020). Therefore, quality control is necessary for accurate quantitative and qualitative identification of MP during the entire sample processing (Foekema et al., 2013).

In order to reduce the overestimation and underestimation of the results, the following measures were observed during the entire analysis:

- Samples were handled in a clean room, in a cabinet with laminar flow and controlled air circulation. During the analyses in the laboratory, doors and windows were closed and maximum of two people stayed in the laboratory.
- Laboratory accessories and equipment for sampling and analysis were made of glass or stainless steel, the use of plastic was completely avoided. Before manipulation, laboratory dishes were washed three times with deionized water, dried and stored covered with clean aluminum foil.
- Avoid wearing synthetic clothing, nitrile gloves and 100 % cotton laboratory coats were used.

– Work surfaces and analytical materials were regularly cleaned with high-quality and pure ethanol and deionized water.
– In order to prevent the loss of MP, the walls of the laboratory dishes, in which the samples were located, were rinsed several times with deionized water.
– Filter papers and Petri dishes were examined under a microscope before use.
– The filters are covered with glass covers during observation under the microscope.

Blanks (negative controls) were included in the study during all analyzes and series, with three replicate blanks per analyzes and series that were treated in parallel with the samples. Intermediate controls were also performed by placing clean Petri dishes with filters next to the sample and then checked for airborne contamination. Fibers (0.4 ± 0.7 MP per filter) were observed in blank samples and intermediate controls. Although a relatively low and negligible concentration was recorded in procedural blank samples (Simon-Sánchez et al., 2019; Jiang et al., 2019), all results were corrected according to the level of contamination measured during sample processing and analysis to compensate for external contamination.

A total of 1.5 % of the chemically analyzed particles were identified by FTIR as not plastic (polymeric) origin. Non-plastic particles (cellulose, non-organic and organic components) were not considered or shown in the results, and all results were corrected in relation to the chemically identified abundance of non-plastic (polymeric) particles.

By correcting all the results in relation to the results of the control tests and the results of the chemical identification of MP, overestimation of the results was prevented, and quantitatively and qualitatively more precise and reliable results were obtained.

3.7 Statistical Analysis

The statistical program PRIMER v7 with PERMANOVA+ software was used to assess the abundance of MP in the inshore surface sediment samples of the investigated freshwater ecosystems in relation to various factors. Before statistical processing, the data were pre-treated using the square root, then the data were transformed using the Bray-Curtis similarity matrix. The following methods were used:

- ***Principal coordinates analysis – PCO*** is a multidimensional statistical method that aims to display as accurately as possible the relationship between variables, the way in which this relationship is determined, as well as the similarities between variables in the original space. The structure of the analyzed data represents a two-dimensional matrix of similarity (or distance) in the coordinate axis for the analyzed variables. The coordinate axis consists of two main components (PCO1 and PCO2). The first principal component (PCO1) takes into account most of the variability in the data, while PCO2 takes into account possible residual variability. The results of the PCO analysis are interpreted based on the contribution or evaluation of the variables in the components (PCO1 and PCO2) (Anderson et al., 2008). PCO analysis calculates the covariance matrix in order to examine all existing relationships through their different values. Correlation is observed when covariance has a positive sign, otherwise they are inversely correlated (Anderson, 2017). PCO analysis examined the degree of similarity and abundance of MP as well as potential sources of MP in relation to the different examined water bodies (rivers/lake), locations and sampling season of inshore surface sediment of freshwater ecosystems.
- ***Cluster analyses – CA*** is a multivariate statistical technique for grouping variables (sediment samples) into meaningful and specific categories (clusters) based on similarities or differences in observed variable values (Sinharay, 2010; Cutillo, 2018). Data classified (joined) in the same cluster indicate the greatest similarity. The clustering procedure can be hierarchical or non-hierarchical. The hierarchical method, which was applied in the dissertation, includes the measurement of different variables for each sample. These variables are then compared between subjects, and clusters are derived in such a way as to minimize the differences between members within a cluster, as well as to maximize the differences between samples that belong to different clusters. Each category (cluster) can then be further divided into lower order categories (subclusters) (McIntosh et al., 2010).
- ***Permutational multivariate analysis of variance – PERMANOVA*** is a non-parametric multivariate statistical permutation test in the space of the chosen measure of diversity according to a given design, with p-values obtained using appropriate permutation techniques. The method was developed in order to perform classical partitioning, thus enabling testing and estimation of main effect sizes, interactions, hierarchical structures,

random components in mixed models, while at the same time retaining important robust statistical data properties of non-parametric multivariate methods. P-values were obtained through the Monte Carlo test of random draws from the asymptotic permutation distribution (Anderson et al., 2008), which shows how many unique values of the test statistic were permuted in order to detect significant differences or correlations. PERMANOVA does not systematically perform all permutations, but displays a random subset of them, and the number of unique values is sufficient to make clear and precise conclusions using the resulting permutation p-value. A Monte Carlo test can involve thousands or tens of thousands of recalculations or permutations (Clarke and Gorley, 2015). Statistical significance is indicated for $p < 0.05$.

RESULTS AND DISCUSSION

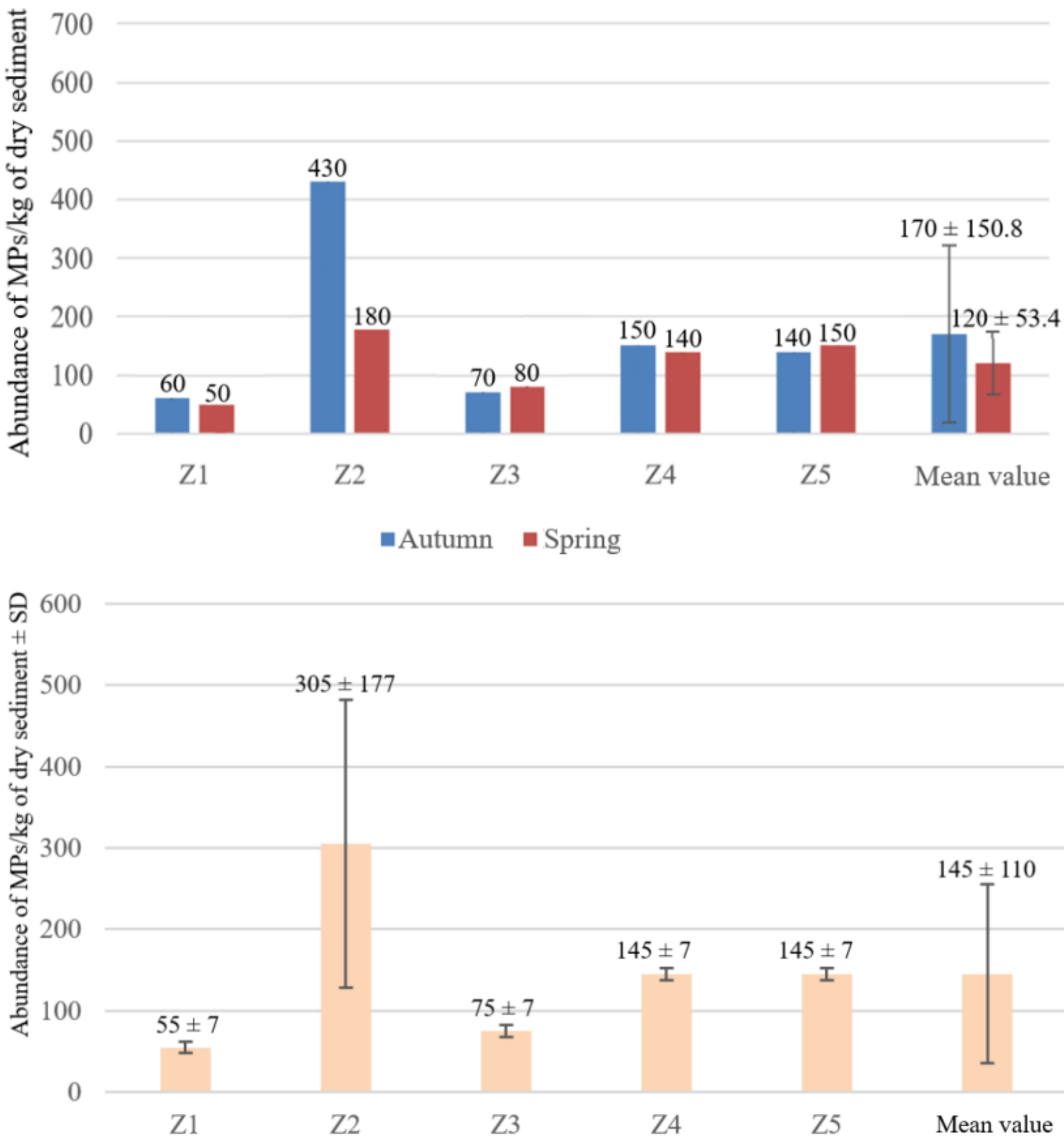
4. ABUNDANCE OF MICROPLASTICS IN RIVER AND LAKE SEDIMENTS

4.1 Zeta

MP was identified in all examined samples of inshore sediment of the Zeta river. The abundance of MP in the inshore sediment of the Zeta river in relation to the locations and sampling season, as well as the total mean abundance of MP at the investigated locations during the entire research period is shown in Figure 4.1.

The total mean abundance of MP in the inshore sediment of the Zeta river at all investigated locations was 170 ± 150.8 MP/kg dry sediment during the autumn season and 120 ± 53.4 MP/kg dry sediment during the spring sampling season with a value range of 50–430 MP/kg dry sediment, Figure 4.1a. The abundance of MP in the inshore sediment of the Zeta river did not differ significantly in relation to the sampling season, except at location Z2, where a significantly higher abundance of MP was recorded during the autumn season compared to the spring sampling season, Figure 4.1a.

The abundance of MP in the inshore sediment of the Zeta river at all investigated locations during the entire research period was in the following order: Z2 > Z4 > Z5 > Z3 > Z1, with a total mean abundance of 145 ± 110 MP/kg of dry sediment (Figure 4.1b). At location Z1, which is located near the source of the Zeta river, as well as at location Z3, which is located at the re-source of Zeta (after plunging), a lower abundance of MP was recorded. The mentioned locations are characterized by low population density, i.e. reduced influence of anthropogenic activities. At location Z2, which is located in the center of Nikšić, which is characterized by significant anthropogenic influences, the highest abundance of MP was recorded. At location Z2, during sampling, the largest amount of waste material of different origins was recorded, Figure 3.4. The medium abundance of MP in the Zeta inshore sediment was recorded at location Z4, which is located in the center of Danilovgrad, and at location Z5, which is located at the entrance to Podgorica near the point where the Zeta flows into Morača river. Both locations are under the influence of various anthropogenic activities such as: wastewater, inadequate management of municipal solid waste, activities of small industries, restaurants, as well as agricultural activities.



a)

b)

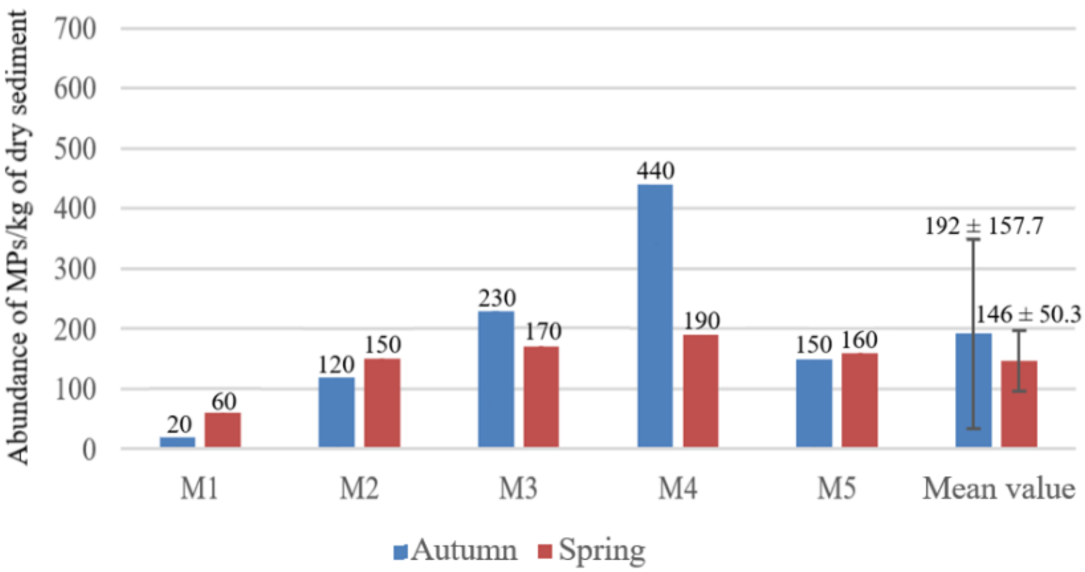
Figure 4.1 Representation of MP abundance in sediment of the Zeta river in relation to a) location and sampling season and b) total mean abundance of MP at the investigated locations at entire research

4.2 Morača

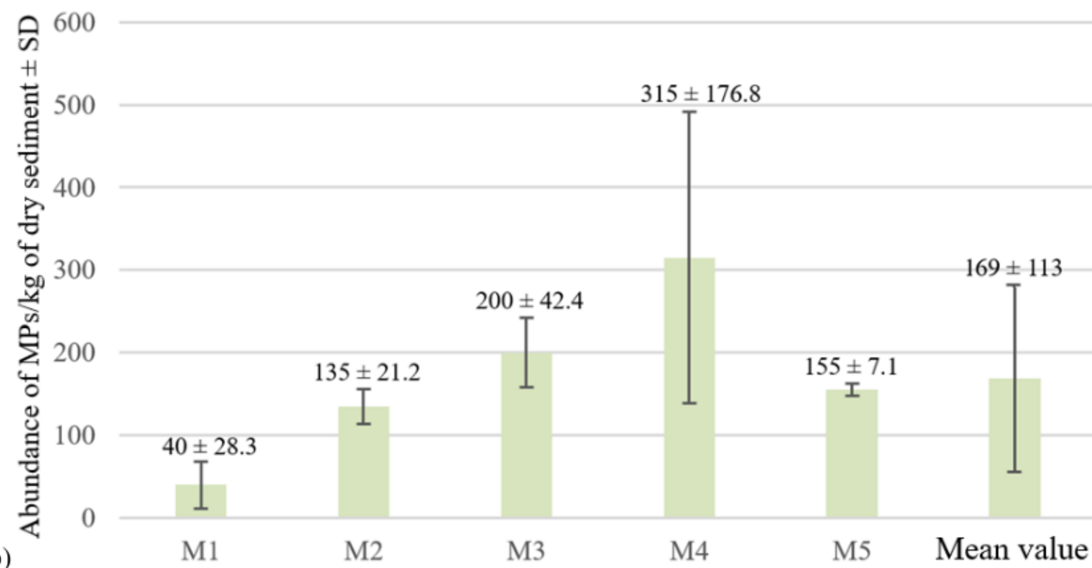
MP was identified in all examined samples of inshore sediment of the Morača river. The abundance of MP in the inshore sediment of the Morača river in relation to the locations and sampling season, as well as the total mean abundance of MP at the investigated locations during the entire research period is shown in Figure 4.2.

The total mean abundance of MP in the inshore sediment of the Morača river at all investigated locations was 192 ± 157.7 MP/kg of dry sediment during the autumn season and 146 ± 50.2 MP/kg of dry sediment during the spring sampling season with a value range of 20–440 MP/kg of dry sediment, Figure 4.2a. At locations M1 and M2, a higher abundance of MP was recorded during the spring season, and at locations M3 and M4 during the autumn sampling season, Figure 4.2a. The abundance of MP in the inshore sediment of the Morača river did not differ significantly in relation to the sampling season at location M5, Figure 4.2a.

The abundance of MP in the inshore sediment of the Morača river at all investigated locations during the entire research period was in the following sequence: M4 > M3 > M5 > M2 > M1, with a total mean abundance of 169 ± 113 MP/kg of dry sediment, Figure 4.2b. At location M1, which is located at the entrance to Podgorica, which is characterized by reduced anthropogenic influence, a lower abundance of MP was recorded. At locations M2 and M3, which are located in the center of Podgorica, and M5, which is located at the exit from Podgorica, a medium abundance of MP was recorded. Locations M2 and M3 are characterized by high population density, as well as a high degree of various anthropogenic activities (high population density, inadequate management of solid municipal waste, activities of small industries, restaurants, as well as agricultural activities), while location M5, in addition to the mentioned anthropogenic activities, is also characterized by direct inflow of the Cijevna river, which may affect the abundance of MP in the sediment. At location M4, which is also located in Podgorica, near the discharge of wastewater from the collector, the highest abundance of MP was recorded.



a)



b)

Figure 4.2 Representation of MP abundance in sediment of the Morača river in relation to a) location and sampling season and b) total mean abundance of MP at the investigated locations at entire research

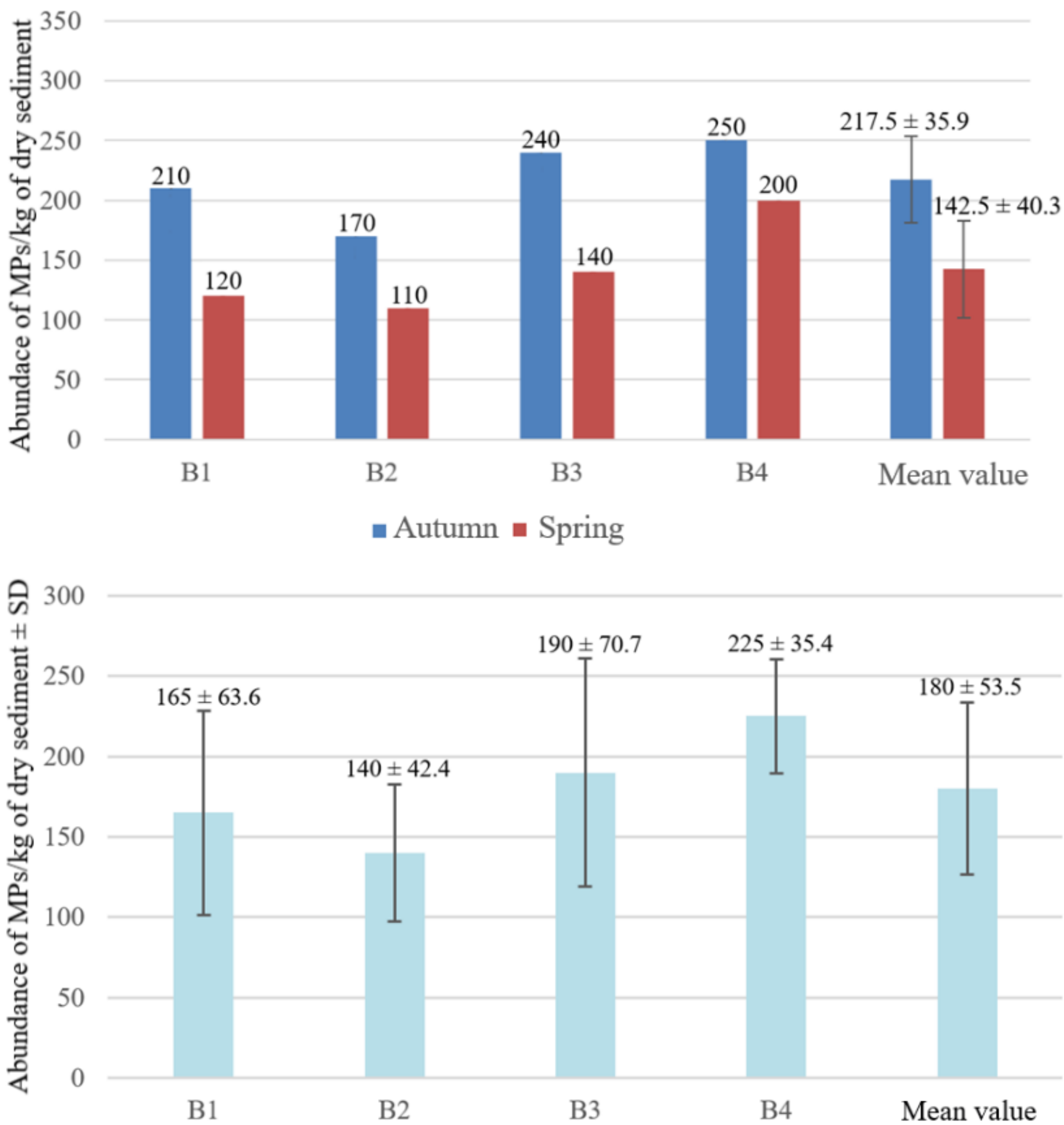
4.3 Bojana

MP was identified in all examined samples of inshore sediment of the Bojana river. The abundance of MP in the inshore sediment of the Bojana river in relation to the locations and the sampling season, as well as the total mean abundance of MP at the investigated locations during the entire research period is shown in Figure 4.3.

In the inshore sediment of the Bojana river, at all investigated locations, the total mean MP abundance of 217.5 ± 35.9 MP/kg of dry sediment during the autumn season and 142.5 ± 40.3 MP/kg of dry sediment during the spring sampling season was recorded with a value range of 110–250 MP/kg of dry sediment, Figure 4.3a. At all investigated locations, a higher abundance of MP was recorded in the inshore sediment of Bojana during autumn, compared to the spring sampling season, Figure 4.3a.

In the inshore sediment of the Bojana river at all investigated locations during the entire research period, MP abundance moved in the following order: B4 > B3 > B1 > B2, with a total mean abundance of 180 ± 53.5 MP/kg of dry sediment, Figure 4.3b.

A lower abundance of MP was observed at location B2, which is located before the river island. The mentioned location is characterized by low population density and anthropogenic activities. A slightly higher abundance of MP was recorded at location B1, which is located on the border between Montenegro and Albania, i.e. at the point where the Bojana river enters the territory of Montenegro. The above results indicate that the Bojana river carries with it pollution originating from Albania, where the Bojana river originates (Liubartseva et al., 2016). At locations B3 and B4, which are located at the beginning and end of the right branch of Ada Bojana, the highest abundance of MP was recorded. The mentioned locations represent important tourist centers during the summer months, so they are under the influence of numerous anthropogenic activities.



a) b)

Figure 4.3 Representation of MP abundance in sediment of the Bojana river in relation to a) location and sampling season and b) total mean abundance of MP at the investigated locations at entire research

4.4 Skadar Lake

MP was identified in all examined samples of inshore sediment of Skadar lake. The abundance of MP in the inshore sediment of Skadar lake in relation to the locations and sampling season, as well as the total mean abundance of MP at the investigated locations during the entire research period is shown in Figure 4.4.

During the autumn sampling season, in the inshore sediment of Skadar lake at all investigated locations, the total mean MP content of 145 ± 40.1 MP/kg dry sediment was recorded, and during the spring sampling season 161.7 ± 46.5 MP/kg dry sediment, with the value range amounted to 90–220 MP/kg of dry sediment, Figure 4.4a. The abundance of MP in the inshore sediment of Skadar lake at locations S1, S2 and S4 did not differ significantly in relation to the sampling season, Figure 4.4a. At location S3, near the Morača river flows into Skadar lake, and S6, a higher abundance of MP was recorded during the spring sampling season, when the inflow of inland waters is greater, while at location S5 a higher abundance of MP was recorded during the autumn sampling season (Figure 4.4a).

In the inshore sediment of Skadar lake at all the investigated locations during the entire research period, abundance of MP moved in the following sequence: S3 > S4 > S5 > S6 > S1 > S2, with a total mean abundance of 153.4 ± 42.7 MP/kg of dry sediment, Figure 4.4b. Location S3, where the highest abundance of MP was recorded, is located near the place where the Morača river flows into Skadar lake. The Morača river with its tributaries is the most important inflow (source) of water into Skadar lake. Wastewater from the municipalities of Nikšić, Danilovgrad and Podgorica is discharged into the Skadar lake via the Morača river, which can significantly affect the quality and pollution of the Skadar lake, as well as the abundance of MP in the lake. Lakeside restaurants, fishing and tourist activities can also contribute to the plastic/MP pollution of this location. Location S4, located opposite location S3, is also characterized by a high abundance of MP. The mentioned location is characterized by a smaller population, the abundance of a couple of restaurants along the shore of the lake, as well as fishing activities. However, it is considered that most of the MP pollution at location S4 originates from location S3, therefore that is was brought by water currents, as a result of the inflow of water from the Morača river.

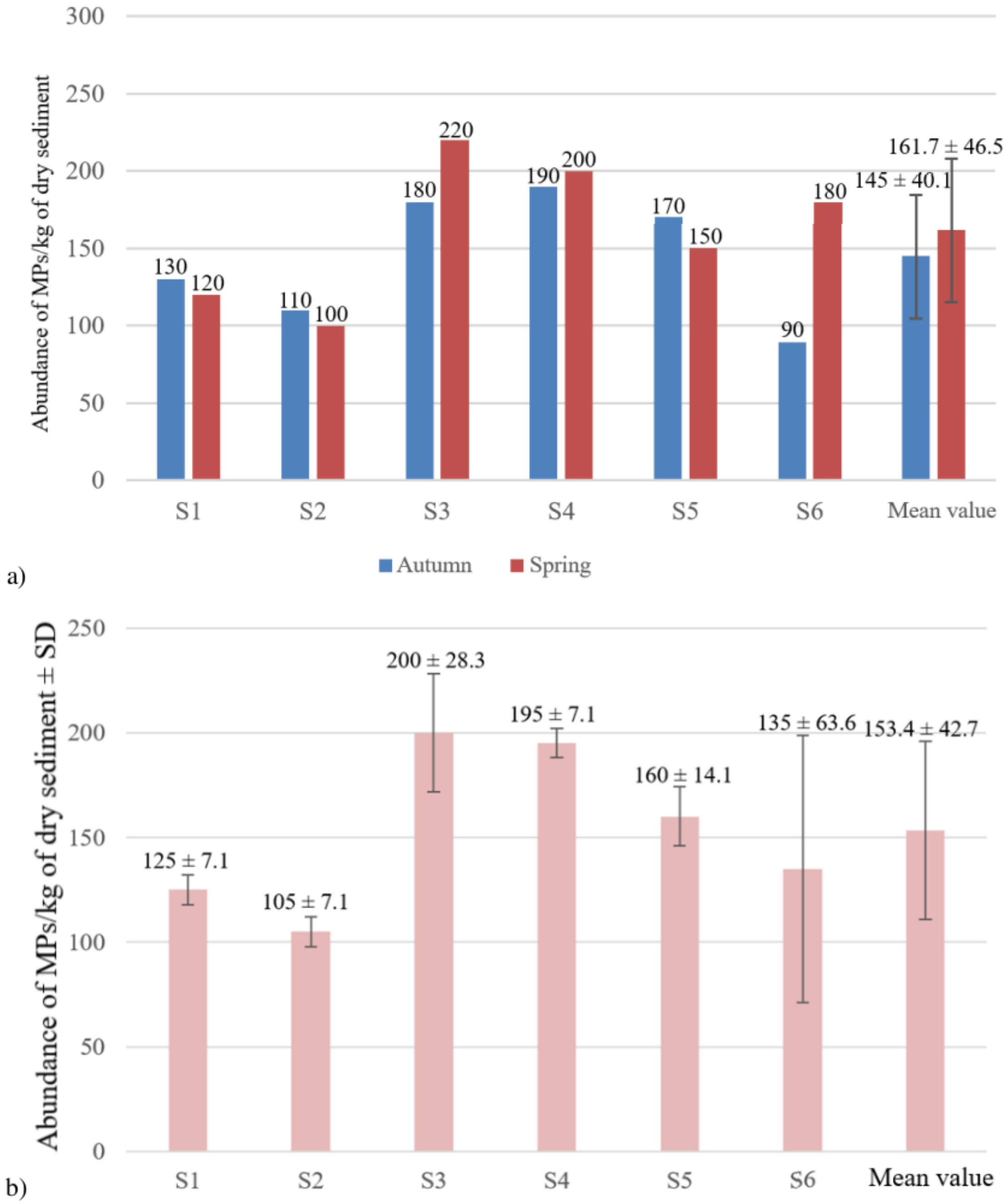


Figure 4.4 Representation of MP abundance in sediment of the Zeta river in relation to a) location and sampling season and b) total mean abundance of MP at the investigated locations at entire research

Location S5 is characterized by a medium abundance of MP. This location is a transit station for a cruise on Skadar lake and a famous picnic spot for its cultural and historical monuments, monasteries and fortifications. It is considered that the sources of MP at this location are mostly wastewater, considering the dysfunctionality of the WWTP of the Municipality of Bar located at this location, so wastewater from the territory of Bar is discharged untreated into Skadar lake (Government of Montenegro, 2019). In addition to wastewater, significant sources of MP in this location are associated with higher population density compared to other locations, problems with solid waste, the abundance of restaurants, tourist and fishing activities. Location S6, which is located near the border with Albania, is characterized by a reduced population density, a huge sandy beach, fish restaurants, fishing and tourist activities, which is why the medium abundance of MP was justifiably observed. At location S1, a significantly lower abundance of MP was recorded than expected, given that the WWTP located near this location is dysfunctional, so wastewater from the territory of Cetinje is discharged untreated into Skadar lake (Government of Montenegro, 2019). Due to its natural, cultural and historical characteristics, this place becomes a significant touristic attraction in the vicinity of Skadar lake, so apart from wastewater, the abundance of restaurants, tourist and fishing activities can be sources of pollution of this location with plastic/MP. At location S2, which represents an authentic fishing village on Skadar lake, the lowest abundance of MP was recorded, which is in accordance with the expected results because the mentioned location is characterized by the least impact of anthropogenic activities.

4.5 Comparative Analysis of the Results of the Abundance and Sources of Microplastics in the Studied Rivers and Lake

CA analysis was used to group and present the abundance of MP in the surface inshore sediment samples of rivers and lake, during the entire research period, in relation to the examined water bodies, locations and sampling season, Figure 4.5.

In Figure 4.5, ten clusters can be observed, where their connection is caused by the level of abundance of MP in relation to the examined locations, water bodies and sampling season. The first cluster is characterized by the lowest abundance of MP (20 MP/kg of dry sediment), while the second cluster is characterized by the highest abundance of MP (430–440 MP/kg of dry sediment).

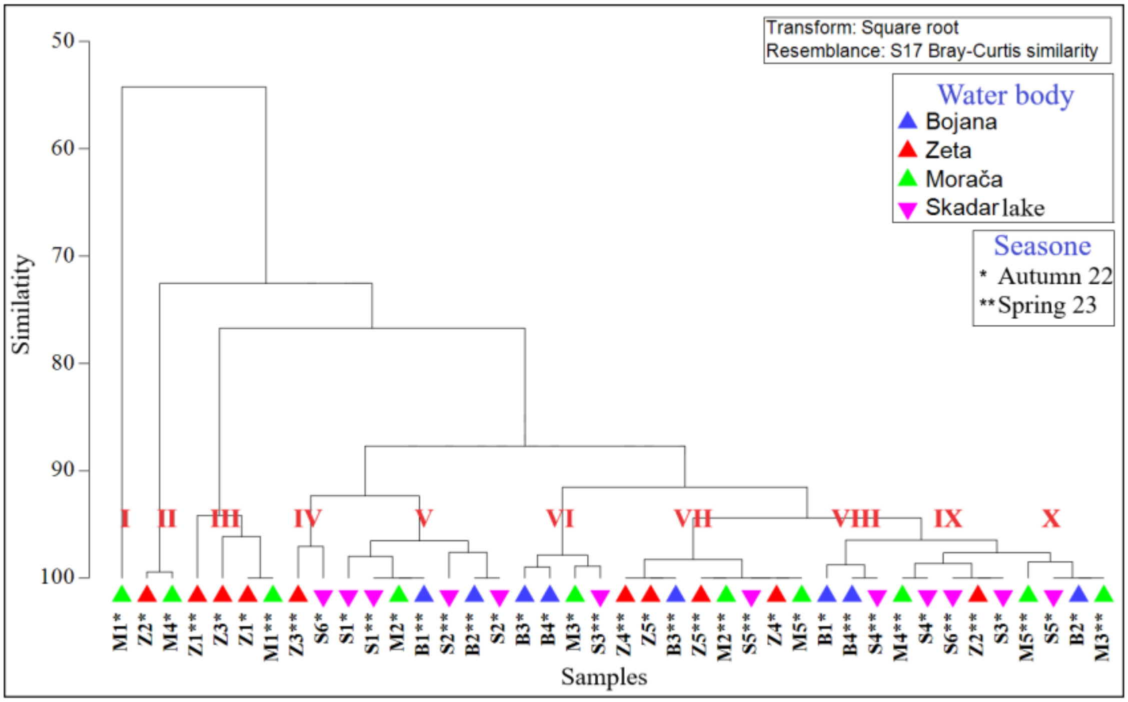


Figure 4.5 Cluster analysis of the MP abudance in sediment samples of rivers and lake during the entire research period, in relation to the examined water bodies, locations and sampling season

The connection of the other clusters is characterized by the following levels of MP abundance: III cluster 50–70 MP/kg of dry sediment; IV cluster 80–90 MP/kg of dry sediment; V cluster 100–130 MP/kg of dry sediment; VI cluster 220–250 MP/kg of dry sediment; VII cluster 140–150 MP/kg of dry sediment; VIII cluster 200–210 MP/kg of dry sediment; IX cluster 180–190 MP/kg dry sediment and X cluster 160–170 MP/kg dry sediment, Figure 4.5. The results of PERMANOVA and the Monte Carlo test indicate that there is no significant statistical correlation in the level of abundance of MP in relation to the examined water bodies, locations and sampling season ($p > 0.05$).

The higher abundance of MP in the spring sampling season may be a consequence of greater inflow of inland water, while the higher abundance of MP in the autumn sampling season may be a consequence of greater anthropogenic influences and activities during the summer season, which is in accordance with the study by Zeri et al. (2018).

The abundance of MP in the inshore sediments of the examined water bodies of the Adriatic basin was in the following sequence: Bojana > Morača > Skadar lake > Zeta. The obtained results justified the expectations, because they reflect different anthropogenic influences on the examined water bodies.

The abundance of MP in river sediments near urban areas as well as near river mouths indicates that population density is a critical factor affecting MP distribution, which is consistent with previous studies (Xu et al., 2020; Firdaus et al., 2020).

The research area includes the central region (Nikšić, Danilovgrad, Podgorica and Cetinje) and the coastal region (Bar and Ulcinj), which together belong to the Adriatic basin of Montenegro. There are four WWTPs in the mentioned area. In several systems, there is a discrepancy between the development of the collector system, the availability and capacity of the appropriate WWTPs. The WWTP in Nikšić is almost completely unused, so a large part of Nikšić untreated wastewater, together with partially treated wastewater, is discharged into the Zeta river. The municipality of Danilovgrad does not own a WWTP, but untreated wastewater from Danilovgrad is directly discharged into the Zeta river. The WWTP in Podgorica has insufficient capacity for wastewater collected within that agglomeration, as Podgorica is the city with the highest population density, so more than half of Podgorica wastewater inflow is discharged untreated into the Morača river. The WWTP in Cetinje (Rijeka Crnojevića) and Bar (Virpazar) is dysfunctional, so wastewater from the territory of Cetinje and Bar is discharged untreated into Skadar lake (Government of Montenegro, 2019). On the other hand, Skadar lake and the Bojana river are water bodies located on the territory of Montenegro and Albania. Barović et al. (2021) state that the pollution of Skadar lake and the Bojana river is an international problem from several directions, from Albania and Montenegro as well as from Kosovo and Macedonia by the Drim river, one branch of which flows into Bojana. Also, Bojana flows out of Skadar lake, so the water quality of Bojana depends on the degree of pollution of Skadar lake. On the basis of the above, it is concluded that wastewater from Nikšić, Danilovgrad, Podgorica, Cetinje and Bar is discharged into Skadar lake, from the territory of Montenegro, which significantly affects the quality and pollution of Bojana, as well as the abundance of MP. The influence of Albania on the pollution of Skadar lake and the Bojana river should not be ignored, as many authors point out (Barović et al., 2018, 2021; Krivokapić, 2021).

Numerous cottages and restaurants were built along the course of the Bojana and their wastewater flows directly into the river (Barović et al., 2021). Pantelić et al. (2020) state that large amounts of pollutants reach the Adriatic Sea via Skadar lake and the Bojana river, which is confirmed by this study. We should not ignore the fact that the Bojana river delta has been declared a protected area, ranking among the most important wetlands in the Eastern Mediterranean (Petković and Sekulić, 2018), and that Skadar lake is a national park, a protected and internationally recognized important bird area, included in the Ramsar List of Wetlands of International Importance and nominated as an EMERALD area (Barović et al., 2018; Krivokapić, 2021), so the preservation of these water ecosystems is a priority. Due to the aforementioned facts, the Bojana river and Skadar lake represent important tourist destinations, which can also affect the increased abundance of MP.

On the other hand, poor waste management practices in Montenegro, such as the lack of necessary trash cans and illegal accumulation (dumping) of waste near the examined water bodies, contribute to a higher prevalence of MP. Fishing and aquaculture can also be an important reason for the abundance of MP in Skadar lake, because synthetic fibers are used in both activities, and the aforementioned activities are more prevalent in Skadar lake, while fishing activities are not significantly represented in the examined rivers (Simon-Sánchez et al., 2019; Yuan et al., 2019). There are no large-scale industrial activities along the studied rivers and lake, so industrial activities cannot be considered important sources of MP in the investigated aquatic ecosystems.

In this study, the factors that can be related to the sources, abundance and distribution of MP in inshore sediments of rivers and lake of the Adriatic basin were identified: hydrodynamic and ecological conditions; population density, touristic, fishing and agricultural activities, wastewater discharge, inadequate solid waste management, as well as cross-border pollution. Similar observations were made by Jiang et al. (2019) in their study.

The total mean abundance of MP during the entire study in the studied rivers and lake of the Adriatic basin was 160.5 ± 83.3 MP/kg of dry sediment. This study indicates the indirect influence of the Zeta and Morača rivers and the Skadar lake, and the direct influence of the Bojana river on the abundance of MP on the Montenegrin coast. Kaiser and Forenbacher (2016) indicate that the Adriatic current flows along the coast of Albania towards Montenegro and Croatia, all the way to the tip of the Istrian peninsula, where it changes direction. This way of movement of currents in the Adriatic Sea may indicate that the rivers belonging to the Adriatic Sea basin, together with the Skadar lake, influence the abundance of MP on the Montenegrin coast. Based on previous research that examined the abundance of MP on the Montenegrin coast of the Adriatic Sea (Bošković et al., 2021, 2022a, 2022b), it can be concluded that the inflow of land waters from the Adriatic basin of Montenegro in this study contributes to the increase in the abundance of MP on the Montenegrin coast. The Monte Carlo test revealed that there is a significant correlation in MP concentration among river and lake sediments and sea sediments ($p < 0.05$). The concentration of MP in open sea locations in previous studies examining the abundance and distribution of MP on the Montenegrin coast ranged between 120-1730 MP/kg of dry sediment (Bošković et al., 2021, 2022a, 2022b, 2022c). The confluence of the Bojana river with the Adriatic Sea is located in the immediate vicinity of the location of Ada Bojana, where it was also established that the inflow of fresh water could be one of the main sources of MP in the investigated location, which was confirmed by this study. In this context, Zeri et al. (2018) state that the inflow of terrestrial waters is one of the significant sources of MP in the seas and oceans. Liubartseva et al. (2016, 2018, 2019) state that the Bojana river carries plastic from the territories of Montenegro and Albania and that it is the second largest terrestrial source of plastic in the entire Adriatic, surpassed only by the Po river. In this regard, as in this study, the highest average abundance of MP was recorded in the Bojana river, it, together with the Morača and Zeta rivers and Skadar lake, represents one of the most significant sources of MP on the Montenegrin coast, but we should not ignore the fact that the Bojana river brings a large amount of MP from Albania (Liubartseva et al., 2016, 2018, 2019).

4.6 Comparison of the Results of the Abundance of Microplastics in the Inshore Sediments of the Studied Rivers and Lake with Available Data from the Literature

Table 4.1 presents a comparison of the abundance of MP in the inshore sediments of the rivers Zeta, Morača and Bojana in this study in relation to previous available studies on the abundance of MP in river sediments from the region and the world.

By comparison with literature data from the region and around the world, the total mean abundance of MP identified in the sediments of the rivers Zeta, Morača and Bojana (145 ± 110; 169 ± 113 and 180 ± 53.5 MP/kg of dry sediment, respectively) was lower than the values recorded in inshore sediments of rivers: Osa (286 ± 37 MP/kg dry sediment) and Albegna (453 ± 424 MP/kg dry sediment) in Italy (Cannas et al., 2017); Tet in France (258 ± 259 MP/kg dry sediment)

(Constant et al., 2020) and Ebro in Spain (2052 ± 746 MP/kg dry sediment) (Simon-Sanchez et al., 2019), Table 4.1. In contrast, the total mean abundance of MP in the inshore sediments of the investigated rivers in this study was higher than the measured values in the riverbeds of: Ljubljanica (23 ± 25 MP/kg dry sediment) and Kamniška Bistrica (22 ± 20 MP/kg dry sediment) in Slovenia (Matjašič et al., 2022), Table 4.1.

The range (min–max) of abundance of MP identified in the sediments of the Zeta, Morača and Bojana rivers (50–430; 20–440 and 110–250 MP/kg of dry sediment, respectively) was lower than the values recorded in the inshore sediments of the rivers: Ombrone in Italy (45–1069 MP/kg dry sediment) (Guerranti et al., 2017); Rhine (228–3763 MP/kg dry sediment) and Main (786–1368 MP/kg dry sediment) in Germany (Klein et al., 2015) and Antuã in Portugal (18–629 MP/kg dry sediment) (Rodrigues et al., 2018), Table 4.1. In contrast, the range of abundance of MP in the examined river inshore sediments in this study was higher than the values recorded in the inshore sediments of the rivers: Cecina (72–191 MP/kg dry sediment) and Po (0.5–78.8 MP/kg dry sediment) in Italy (Blašković et al., 2018; Atwood et al., 2019), Table 4.1.

Table 4.2 presents a comparison of abundance of MP in the inshore sediment of Skadar lake in this study in relation to previous available studies on abundance of MP in lake sediments from the region and the world.

The total mean abundance of MP identified in the sediment of Skadar lake in this study (153.4 ± 42.7 MP/kg dry sediment) was lower than the values recorded in the inshore sediments of Chiusi lake in Italy (234 ± 85 MP/kg dry sediment) (Fischer et al., 2016); Vesijarvi in Finland (395.8 ± 90.7 MP/kg dry sediment) (Scopetani et al., 2019); Simcoe in the USA (372 ± 346 MP/kg dry sediment) (Felismino et al., 2021); Hampstead Pond in London (539 MP/kg dry sediment) (Turner et al., 2019) and Ontario in Canada (352 MP/kg dry sediment) (Corocran et al., 2015), Table 4.2. In the sediments of the Bizerte Lagoon in Tunisia (7960 ± 6870 MP/kg of dry sediment) (Abidli et al., 2017), a significantly higher abundance of MP was recorded compared to the abundance of MP in the sediment of Skadar lake in this study, Table 4.2. The total mean abundance of MP identified in the sediment of Skadar lake in this study was higher than the values recorded in: Bolosena lake in Italy (112 ± 32 MP/kg dry sediment) (Fischer et al., 2016) and Victoria lake in Africa (75.2 ± 50.0 MP/kg dry sediment) (Egessa et al., 2019), Table 4.2.

Table 4.1 Comparison of the presence, type of polymer, shape, size and color of MP in river sediments found in this study compared to previous studies on the presence of MP in river sediments from the region and the world (MP/kg dry sediment)

River / country	Type of sediment	Min-max / average value	Polymer	Shape	Size (mm)	Color	References
Zeta, Montenegro	Shore river	50-430 / 145 ± 110	PP, PE	Fragments, fibers	0.5-1	Blue, red, clear	
Morača, Montenegro	Shore river	20-440 / 169 ± 113	PE, PET	Fibers	0.5-1	Blue, red, clear	This study
Bojana, Montenegro	Shore river	110-250 / 180 ± 53.5	PP, PE	Fibers	0.5-1	Blue, clear, red	
Ljubljanica, Slovenia	River bed	5-40 / 23 ± 25	PE, PP	Fragments	0-0.99	Colored	Matjašič et al. 2022
Kamniška Bistrica, Slovenia	River bed	10-33 / 22 ± 20	PE, PP	Fragments	0-0.99	Colored	
Ticino, Italy	/	/ 11 ± 7.7	EVA	/	/	/	Winkler et al. 2022
Po, Italy	Shore river	0.5-78.8 /	PE, PP	/	/	/	Atwood et al. 2019
Osa, Italy	Shore river	/ 286 ± 37	/	Fibers	/	Colored	Cannas et al. 2017
Albegna, Italy	Shore river	/ 453 ± 424	/	Fibers	/	Colored	
Ombrone, Italy	Shore river	45-1069 /	/	Fibers	/	Black, white, clear	Guerranti et al. 2017
Cecina, Italy	Shore river	72-191 /	PVC	Fragments, fibers	0.630-1.462	Colored	Blašković et al. 2018
Tisa, Central Europe	River bed	/ 3177 ± 1970	/	Fibers	/	/	Kiss et al. 2021
Rajna, Germany	Shore river	228-3763 /	PE, PP, PS	Fragments	0.630-5.0	Colored	Klein et al. 2015
Majna, Germany	Shore river	786-1368 /	PE, PP, PS	Fragments	0.630-5.0	/	
Elba, Germany	River bed	9-15962 / 2080 ± 4670	PE, PP	Granules, fragments	0.126-5.0	Clear	Scherer et al. 2020
Tet, France	Shore river	7.3-1029 / 258 ± 259	PE, PP	Fragments, fibers	/	/	Constant et al. 2020
Ebro, Spain	Shore river River bed	/ 422 ± 119 / 2052 ± 746	PA, PE	Fibers	0.200-0.500	Clear, orange	Simon-Sanchez et al. 2019
Kelvin, United Kingdom	Shore river	161-432 /	/	Fibers	/	Colored	Blair et al. 2019
Antuã, Portugal	Shore river	18-629 /	PE, PP	Fragments	0.055-5.0	Colored	Rodrigues et al. 2018
Brisbejn, Kvinslend, Australia	River bed	10-520 /	PE, PA, PP	Films	< 3.00	Colored	He et al. 2019
Daljao, China	River bed	100-467 / 237 ± 139	PE, PP, PET	Films, fragments	/	/	Xu et al. 2020
Huanpu, China	River bed	133-300 / 170 ± 96	PE, PP, PET	Films, fragments	/	/	
Otava, Canada	River bed	/ 220	/	Fragments, fibers	/	Red, blue	Vermaire et al. 2017
Sen Loren, Canada	River bed	65-7562 / 832 ± 150	/	Granules, fragments	< 400	/	Crew et al. 2020
Solimões, Negro and Amazon, Brazil	River bed	417-8178 /	/	Fibers	0.063–5.0	White, blue, black	Gerolin et al. 2020

Table 4.2 Comparison of the presence, type of polymer, shape, size and color of MP in Skadar lake sediments found in this study compared to previous studies on the presence of MP in lake sediments from the region and the world (MP/kg dry sediment)

Lake / country	Type of sediment	Min-max / average value	Polymers	Shape	Size (mm)	Color	References
Skadar lake, Montenegro	Shore lake	90-220 153.4 ± 42.7	PE, PP	Fibers, fragments	1-3	Blue, red, clear	This study
Bolosena lake, Italy	Coastal	/ 112 ± 32	/	Fragments, fibers	0.3-0.5	/	Fischer et al. 2016
Chiusi lake, Italy	Coastal	/ 234 ± 85	/	Fibers, fragments	0.3-0.5	/	
Bizerte lagoon, Tunisia	Coastal	3000-18000 7960 ± 6870	/	Fibers, fragments	0.3-5.0	Clear, white, blue	Abidli et al. 2017
Lake Tollene, Germany	Surface	/ 1410 ± 822	PE, PET	Fragments	0.063-2.0	/	Hengstmann et al. 2021
Hampstead Pond no. 1, London	Surface	/ 539	PS, PA	Fibers	0.5-1.0	Blue, white, red	Turner et al. 2019
Vesijarvi lake, Finland	Coastal	/ 395.8 ± 90.7	PA, PS, PET	Fibers, fragments	/	/	Scopetani et al. 2019
Poyang lake, China	Surface	54-506 /	PP, PE	Fibers, films	< 0.5	Blue, green, yellow	Yuan et al. 2019
Ulansuhai lake, China	Surface	14-24 /	PE, PET, PP	Granules	< 2.0	Colored	Qin et al. 2019
East Dongting lake, China	Shore lake	180-693 403	PET, PA, PE, PP	Fibers	<0.5, 0.5-1	Clear	Yin et al. 2020
Anchar lake, India	Surface	233-1533 606 ± 360	PA	Fibers	0.3–1, 2-5	White, red, blue	Neelavannan et al. 2022
Kodaikanal lake, India	Surface	/ 28.31 ± 5.29	PE, PP	Fibers, fragments	3-5	/	Laju et al. 2022
Rawal lake, Pakistan	Coastal	/ 104	PE,PP	Fibers, fragments	< 1	/	Irfan et al. 2020
Phewa lake, Nepal	Surface	/ 100.5 ± 58.6	PP, PE	Fibers	0.2-1	Clear	Malla-Pradhan et al. 2022
Ontario lake, Canada	Surface	/ 352	PE, PP	Fibers, films	/	Colored	Corocran et al. 2015
Michigan lake, USA	Surface	32.9-6229 /	PP, PS, PE	Granules, fibers	0.125-0.354	Black	Lenaker et al. 2019
Mead lake, USA	Coastal	87.5-1010 /	/	Fibers	0.35-1	/	Baldwin et al. 2020
Simcoe lake, USA	Surface	8.3-1070 372 ± 346	PE, PP	Fibers	> 1.25	Blue, black, red	Felismino et al. 2021
Victoria lake, Africa	Coastal	0.9-239.8 75.2 ± 50.0	PE, PP	Fragments, fibers	1.0-2.0	/	Egessa et al. 2019

5. VISUAL IDENTIFICATION OF MICROPLASTICS IN RIVERS AND LAKE SEDIMENT

Figure 5.1 shows the representative MP particles detected by visual identification in the studied samples of surface inshore sediments of rivers and lake.

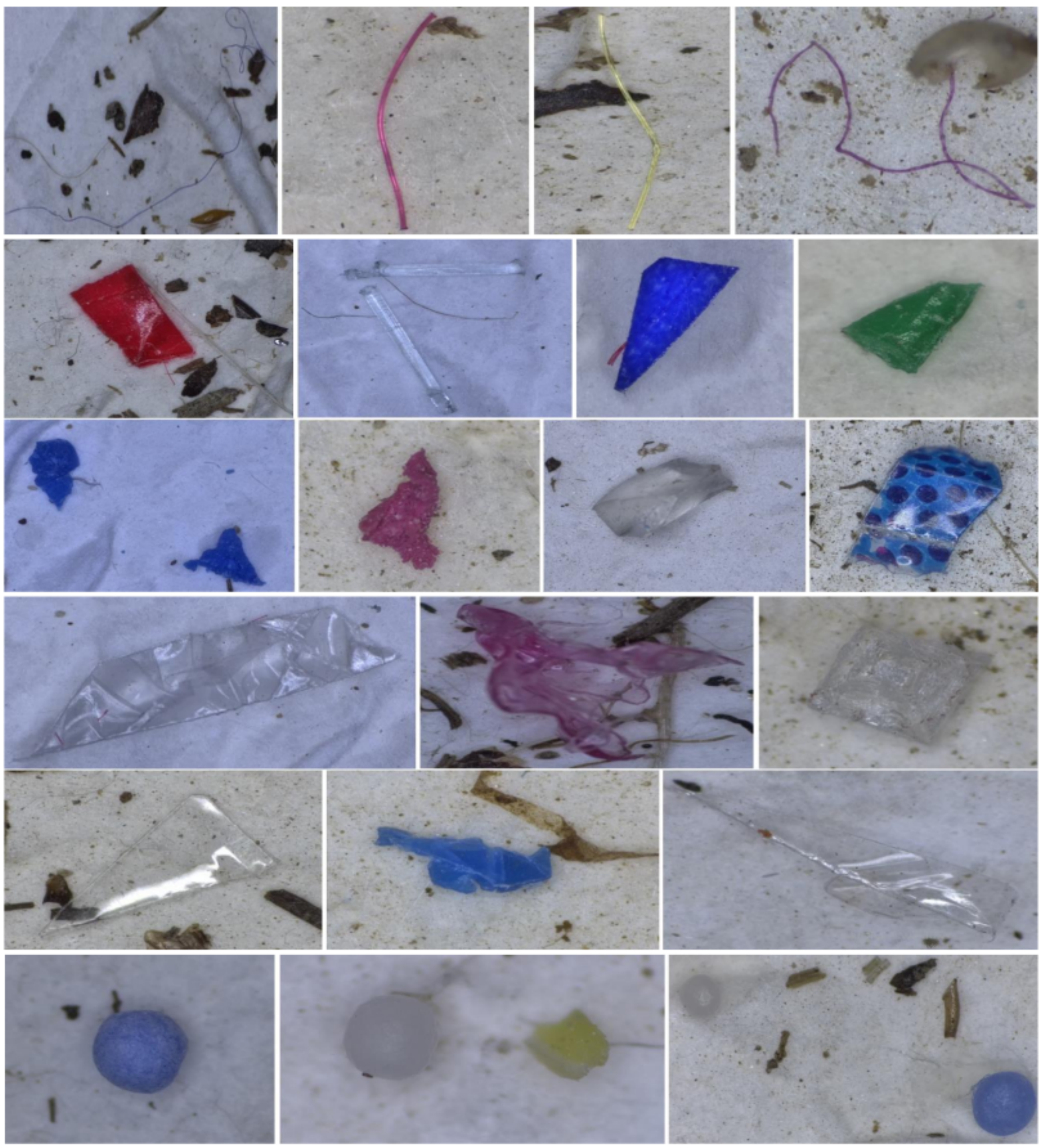


Figure 5.1 Representative MP particles detected by visual identification in the examined sediment samples of rivers and lake

5.1 Zeta

The percentage abundance of the shape, color and size of MP in the inshore sediment of the Zeta river in relation to the sampling season and during the entire research period is shown in Figure 5.2.

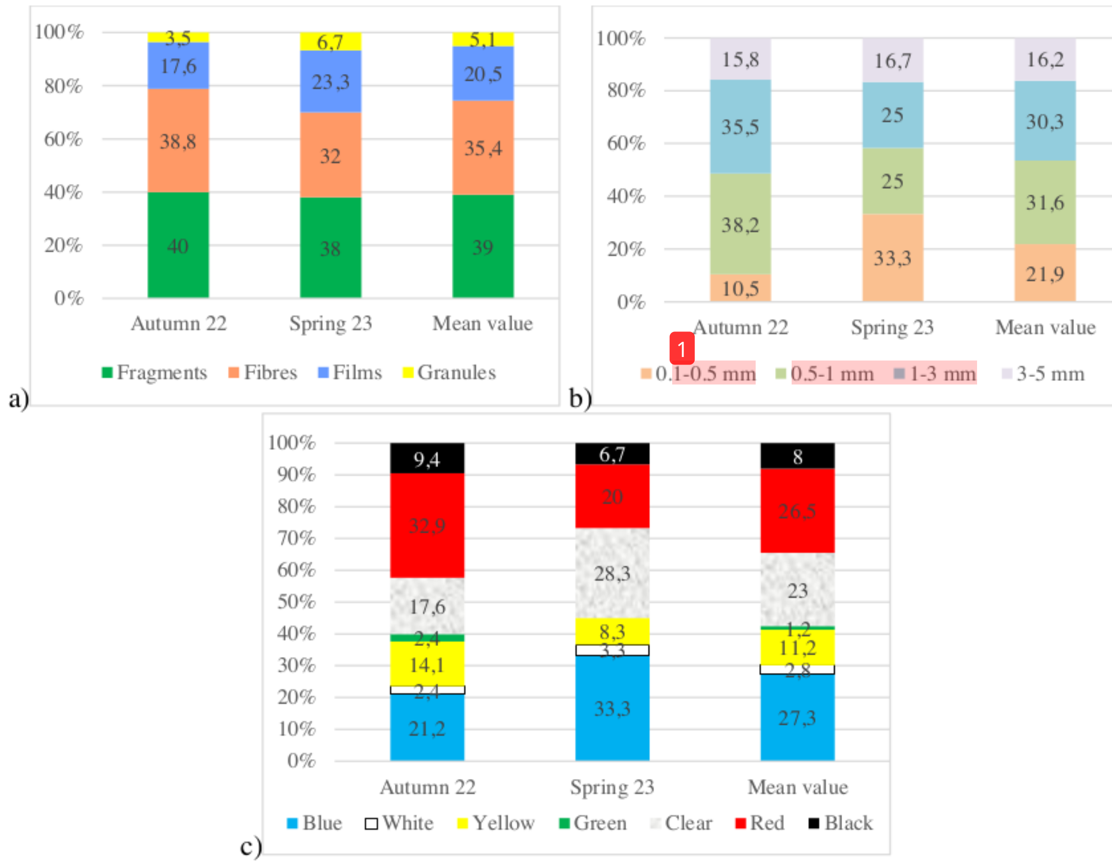


Figure 5.2 Figure 5.2 Percentage MP abundance of a) shape, b) size and c) color in sediment of the Zeta river based on the sampling season and during the entire research period

The abundance of MP shape in the inshore sediment of the Zeta river during the entire research was in the following order: fragments (39 %) > fibers (35.4 %) > films (20.5 %) > granules (5.1 %), Figure 5.2a. It is possible to see from the Figure 5.2a that the total abundance of MP shape in the inshore sediment of the Zeta river did not differ significantly in relation to the sampling season.

The abundance of MP size in the inshore sediment of the Zeta river during the entire study was in the following order: 0.5–1.0 mm (31.6 %) > 1–3 mm (30.3 %) > 0.1–0.5 mm (21.9 %) > 3–5 mm (16.2 %), Figure 5.2b. The abundance of MP size in the inshore sediment of the Zeta river differed in relation to the sampling season, so during the autumn sampling season it ranged from: 0.5–1 mm; 1–3 mm; 3–5 mm and 01–0.5 mm, and during the spring sampling season in the following order: 0.1–0.5 mm; 0.5–1 mm; 1–3 mm and 3–5 mm, Figure 5.2b.

The abundance of MP color in the inshore sediment of the Zeta river during the entire study was in the following order: blue (27.3 %) > red (26.5 %) > clear (23 %) > yellow (11.2 %) > black (8 %) > white (2.8 %) > green (1.2 %), Figure 5.2c. The abundance of MP color in the inshore sediment of the Zeta river differed in relation to the sampling season, so during the autumn sampling season it ranged from: red; blue; clear; yellow; black; white and green, and during the spring sampling season with the following sequence: blue; clear; red; yellow; black and white, Figure 5.2c.

5.2 Morača

The percentage abundance of the shape, color and size of MP in the inshore sediment of the Morača river in relation to the sampling season and during the entire research period is shown in Figure 5.3.

The abundance of MP shape in the inshore sediment of the Morača river during the entire study was in the following sequence: fibers (46.3 %) > fragments (28.4 %) > films (13.6 %) > granules (11.8 %), which is similar to the total abundance of MP shape in inshore sediment of the Morača river during the spring sampling season, Figure 5.3a. The total abundance of MP shape in the inshore sediment of the Morača river during the autumn sampling season was in the following sequence: fibers; fragments; granules and films, Figure 5.3a.

The abundance of MP size in the inshore sediment of the Morača river during the entire study was in the following order: 0.5–1 mm (37.2 %) > 1–3 mm (28.1 %) > 3–5 mm (21.7 %) > 01–0.5 mm (13 %), which is similar to the total abundance of MP size in the inshore sediment of the Morača river during the autumn sampling season, Figure 5.3b. During the spring sampling season, the MP size in the inshore sediment of the Morača river was in the following sequence: 1–3 mm; 0.5–1 mm; 3–5 mm and 01–0.5 mm, Figure 5.3b.

During the entire research, the abundance of MP color in the inshore sediment of the Morača river moved in the following order: blue (26.5 %) > red (23.1 %) > clear (22.6 %) > black (11 %) > yellow (8.8 %) > white (4.3 %) > green (3.6 %), Figure 5.3c. The abundance of MP color in the inshore sediment of the Morača river did not differ significantly in relation to the sampling season, Figure 5.3c.

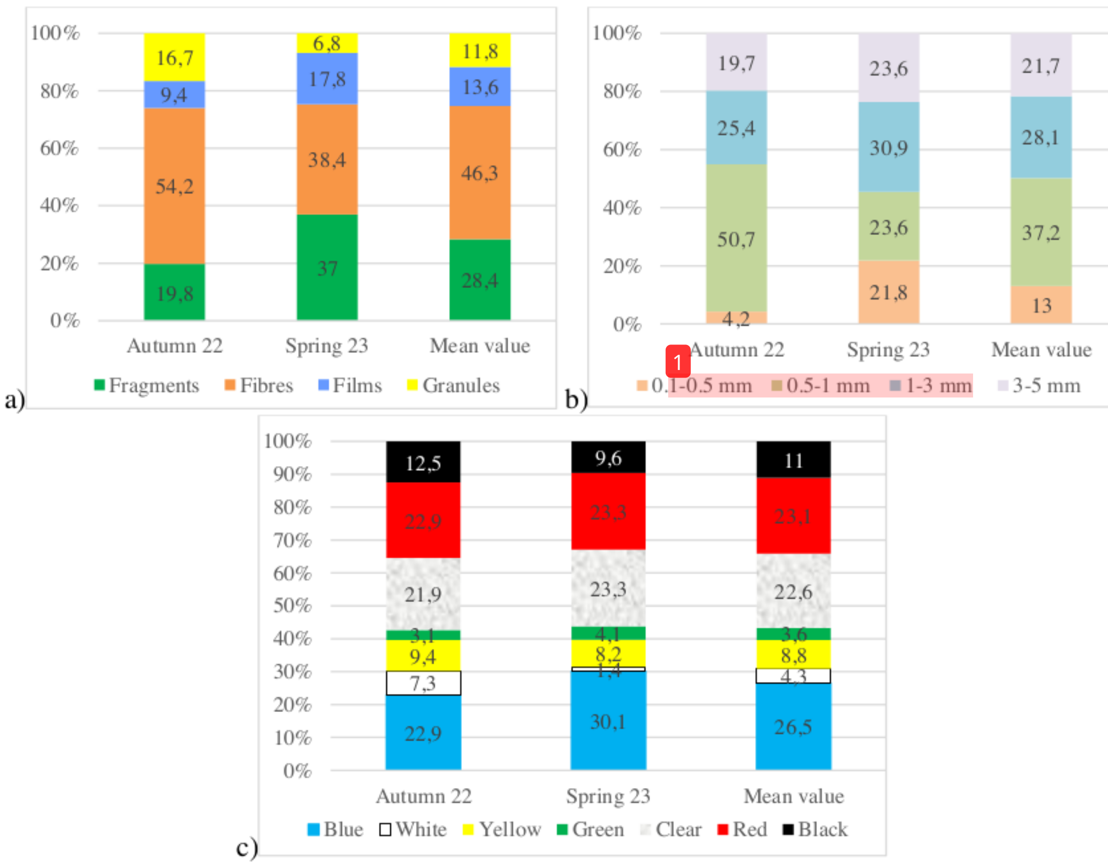


Figure 5.3 Figure 5.2 Percentage MP abundance of a) shape, b) size and c) color in sediment of the Morača river based on the sampling season and during the entire research period

5.3 Bojana

The percentage abundance of the shape, color and size of MP in the inshore sediment of the Bojana river in relation to the sampling season and during the entire research period is shown in Figure 5.4.

During the entire research, the abundance of MP shape in the inshore sediment of the Bojana river moved in the following order: fibers (46.4 %) > fragments (34.2 %) > films (13 %) > granules (6.4 %), Figure 5.4a. The total abundance of MP shape in the inshore sediment of the Bojana river did not differ significantly in relation to the sampling season, Figure 5.4a.

The abundance of MP size in the inshore sediment of the Bojana river during the entire study was in the following order: 0.5–1 mm (37.2 %) > 1–3 mm (30.7 %) > 0.1–0.5 mm (16.2 %) > 3–5 mm (15.9 %), Figure 5.4b. The abundance of MP size in the inshore sediment of the Bojana river differed in relation to the sampling season, so during the autumn sampling season it ranged from: 0.5–1 mm; 1–3 mm; 3–5 mm and 0.1–0.5 mm, and during the spring sampling season in the following sequence: 0.5–1 mm; 0.1–0.5 mm; 1–3 mm and 3–5 mm, Figure 5.4b.

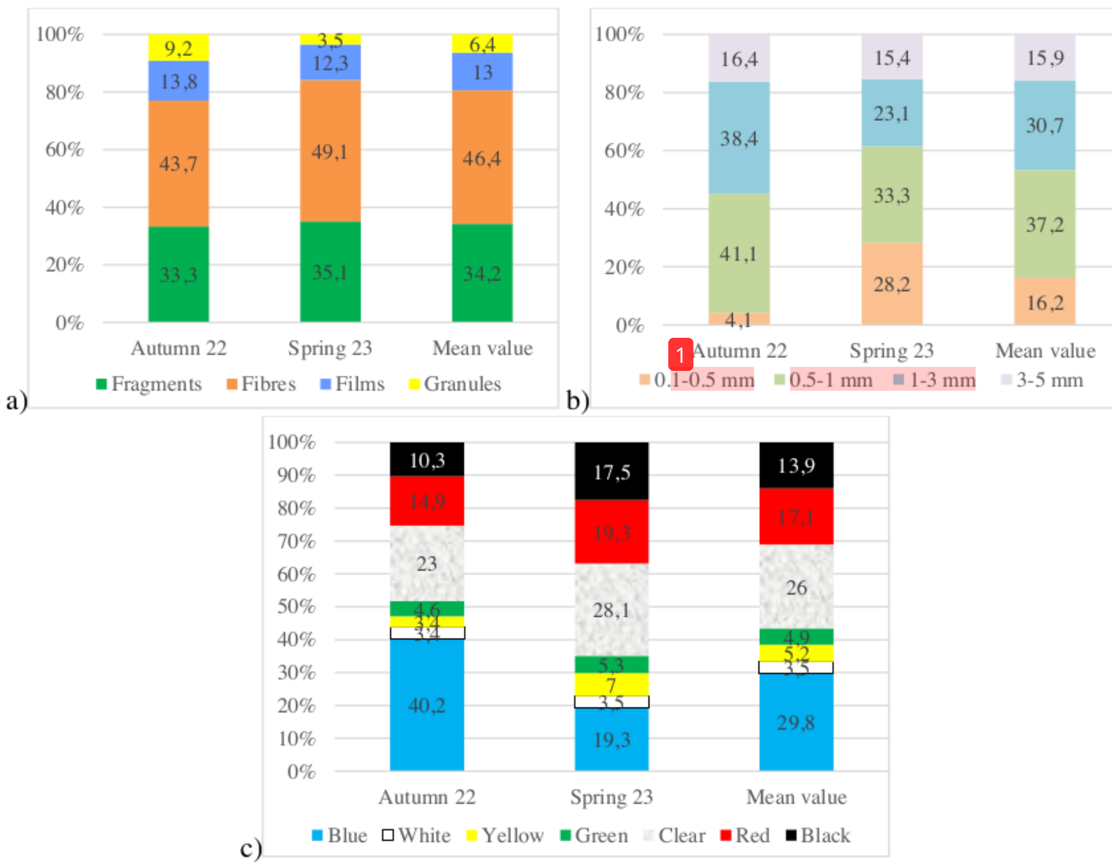


Figure 5.4 Figure 5.2 Percentage MP abundance of a) shape, b) size and c) color in sediment of the Bojana river based on the sampling season and during the entire research period

In the inshore sediment of the Bojana river, during the entire research, the abundance of MP color moved in the following order: blue (29.8 %) > clear (26 %) > red (17.1 %) > black (13.9 %) > yellow (5.2 %) > green (4.9 %) > white (3.5 %), Figure 5.4c. During the autumn sampling season, the abundance of MP color in the inshore sediment of the Bojana river moved in the following sequence: blue; clear; red; black; green; yellow and white, and during the spring sampling season the following: clear; blue; red; black; yellow; green and white, Figure 5.4c.

5.4 Skadar Lake

The percentage abundance of the shape, color and size of MP in the inshore sediment of Skadar lake in relation to the sampling season and during the entire research period is shown in Figure 5.5.

Figure 5.5 Figure 5.2 Percentage MP abundance of a) shape, b) size and c) color in sediment of the Skadar lake based on the sampling season and during the entire research period

The abundance of MP shape in the inshore sediment of Skadar lake during the entire study was in the following order: fibers (41%) > fragments (36%) > films (23%), which is similar to the total abundance of MP shape in the inshore sediment of Skadar lake during the autumn sampling season, Figure 5.5a. The total abundance of MP shape in the inshore sediment of Skadar lake during the spring sampling season was in the following order: fragments; fibers and films, Figure 5.5a. Granules were not identified in the examined sediments of Skadar lake, Figure 5.5a.

In the inshore sediment of Skadar lake, during the entire research, the abundance of MP size was ranged in the following sequence: 1–3 mm (33.3 %) > 0.5–1 mm (28.6 %) > 3–5 mm (24.5 %) > 01–0.5 mm (13.7 %), Figure 5.5b. During the autumn sampling seasons, MP size in the inshore sediment of Skadar lake was ranged in the following sequence: 1–3 mm; 0.5–1 mm; 3–5 mm and 01–0.5 mm, and during the spring sampling season with the following sequence: 1–3 mm; 0.5–1 mm; 0.1–0.5 mm and 3–5 mm, respectively (Figure 5.5b).

During the entire research, the abundance of MP color in the inshore sediment of Skadar lake was in the following order: blue (31.9 %) > clear (26.4 %) > red (21.7 %) > yellow (6.7 %) > black (6.5 %) > green (4.3 %) > white (2.6 %), Figure 5.5c. During the autumn sampling season, the abundance of MP color in the inshore sediment of Skadar lake was in the following oder: blue; clear; red; yellow; black and green, and during the spring sampling season with the following sequence: clear; blue; red; black; white; green and yellow, Figure 5.5c.

5.5 Comparative Analysis of the Results of the Visual Identification of Microplastics

In general, the abundance of MP shape in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) was in the following sequence: fibers (42.4 %) > fragments (34.3 %) > films (17.4 %) > granules (5.9 %). In the studied sediment samples, fibers, followed by fragments, were the most abundant shape of MP.

Figure 5.6 shows the PCO and CA analysis of the abundance of MP shape type in studied surface inshore samples of rivers and lake sediments during the entire research period.

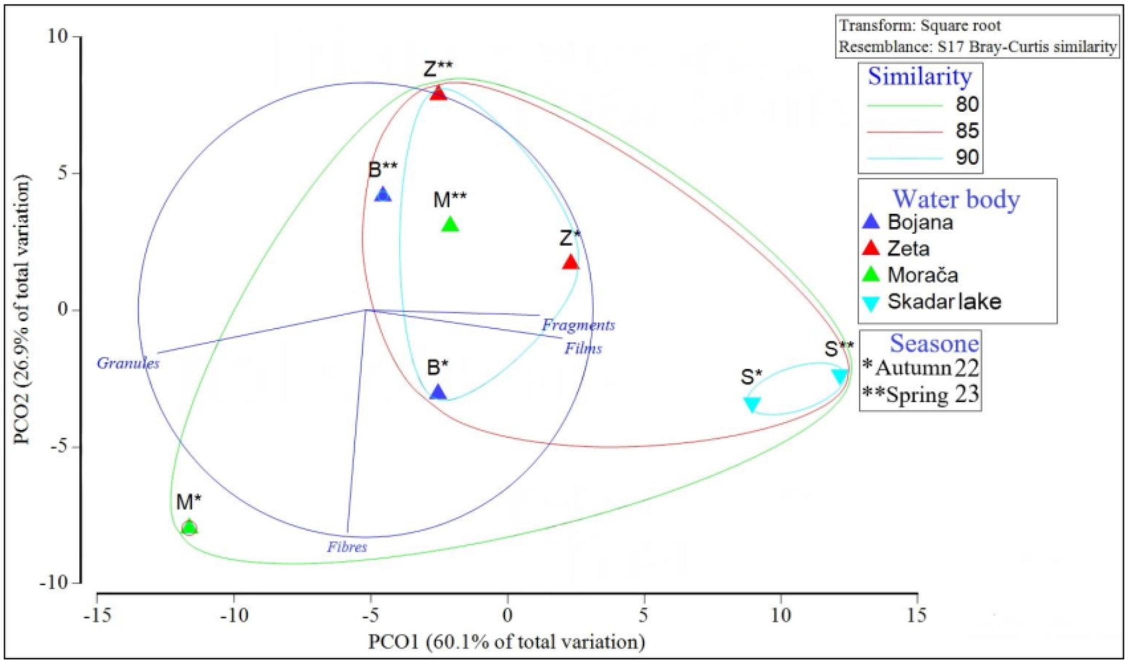


Figure 5.6 PCO and CA analysis of MP shape type abundance in sediment samples of rivers and lake during the entire research period

Figure 5.6 shows two clusters with a mutual similarity of 80 %, Figure 5.6. The first cluster includes surface inshore sediment samples of the Morača river during the autumn sampling season, where the highest abundance of granules and fibers was recorded, and the lowest abundance of fragments and films, Figure 5.6. The second cluster includes three subclusters with mutual similarity of 85 %. The first subcluster includes surface inshore samples of the Bojana river sediment during the spring sampling season, in which a lower abundance of films and fragments was recorded, Figure 5.6. The second subcluster includes surface inshore sediment samples of the rivers Zeta and Bojana during the autumn sampling season and Zeta and Morača during the spring sampling season, and they are characterized by a similar abundance of all types of MP shapes, Figure 5.6. The third subcluster includes the surface inshore sediment samples of Skadar lake during the autumn and spring sampling seasons, which are characterized by the absence of granules and a higher prevalence of films, Figure 5.6. The sum of the two main components of the PCO analysis (PCO1 and PCO2) make up 87 % of the total variations. PCO, PERMANOVA and Monte Carlo test indicate that there is no significant statistical correlation in the abundance of MP shape type in relation to the examined water bodies and the sampling season ($p > 0.05$), Figure 5.6.

In the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake), the overall abundance of MP size ranged in the following sequence: 0.5-1 mm (34.4 %) > 1-3 mm (31.2 %) > 3-5 mm (19.6 %) > 0.1-0.5 mm (14.8 %). The results indicate that MP of the medium size category is the most abundant in the studied sediment samples.

Figure 5.7 shows the PCO and CA analysis of MP size abundance in surface inshore sediment samples of rivers and lake during the entire research period.

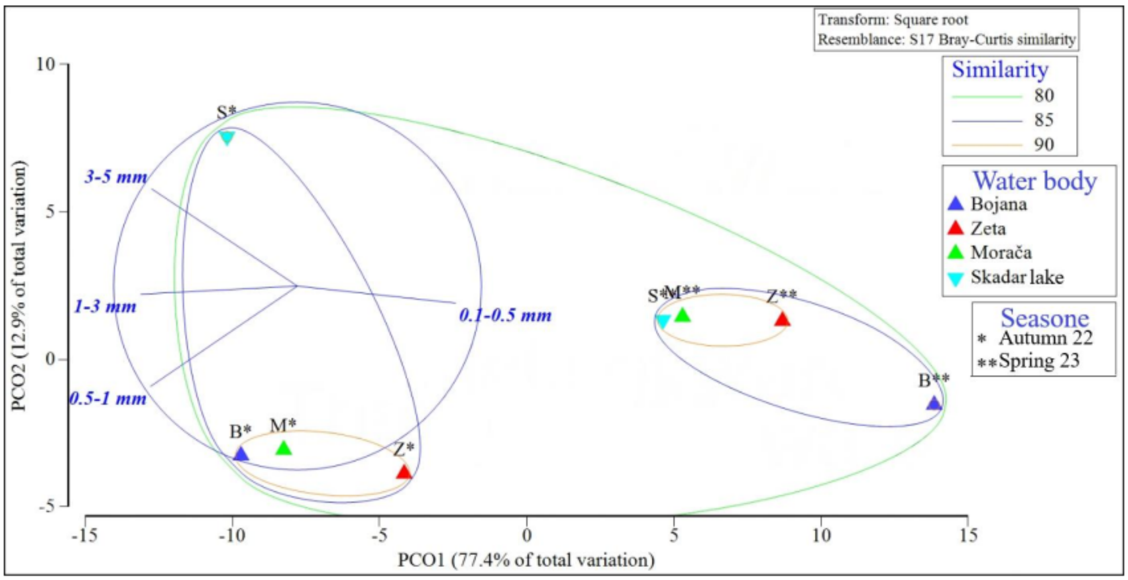


Figure 5.7 PCO and CA analysis of MP size class abundance in sediment samples of rivers and lake during the entire research period

CA analysis indicates the abundance of two clusters with mutual similarity of 80 % and mutual similarity within clusters of 85-90 %, Figure 5.7. The first cluster includes surface inshore sediment samples of rivers and lake sampled during the autumn sampling season, where a higher prevalence of MP size 3-5 mm (Skadar lake) and 0.5-3 mm (Zeta, Morača and Bojana) was recorded compared to the spring sampling season, Figure 5.7. The second cluster includes surface inshore sediment samples of rivers and lake sampled during the spring sampling season, where a higher prevalence of MP size 0.1-0.5 mm was recorded compared to the autumn sampling season, Figure 5.7. The PCO analysis, which represents 90.3 % of the total variation with the sum of the two main components, shows the statistical correlation of the samples based on the abundance of the MP size in relation to the sampling season (PCO1), which is confirmed by both the PERMANOVA and the Monte Carlo test ($p < 0.05$), while the statistical correlation of samples is not observed based on the abundance of MP size in relation to the examined water bodies (Monte Carlo test, $p > 0.05$), Figure 5.7.

The total abundance of MP color in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) was in the following order: blue (29 %) > clear (24.1 %) > red (22.3 %) > black (9.7 %) > yellow (7.9 %) > green (3.6 %) > white (3.4 %). Blue, clear and red are the most abundant color categories in the studied sediment samples.

PCO and CA analysis of MP color abundance in surface inshore sediment samples of rivers and lake during the entire research period is shown in Figure 5.8.

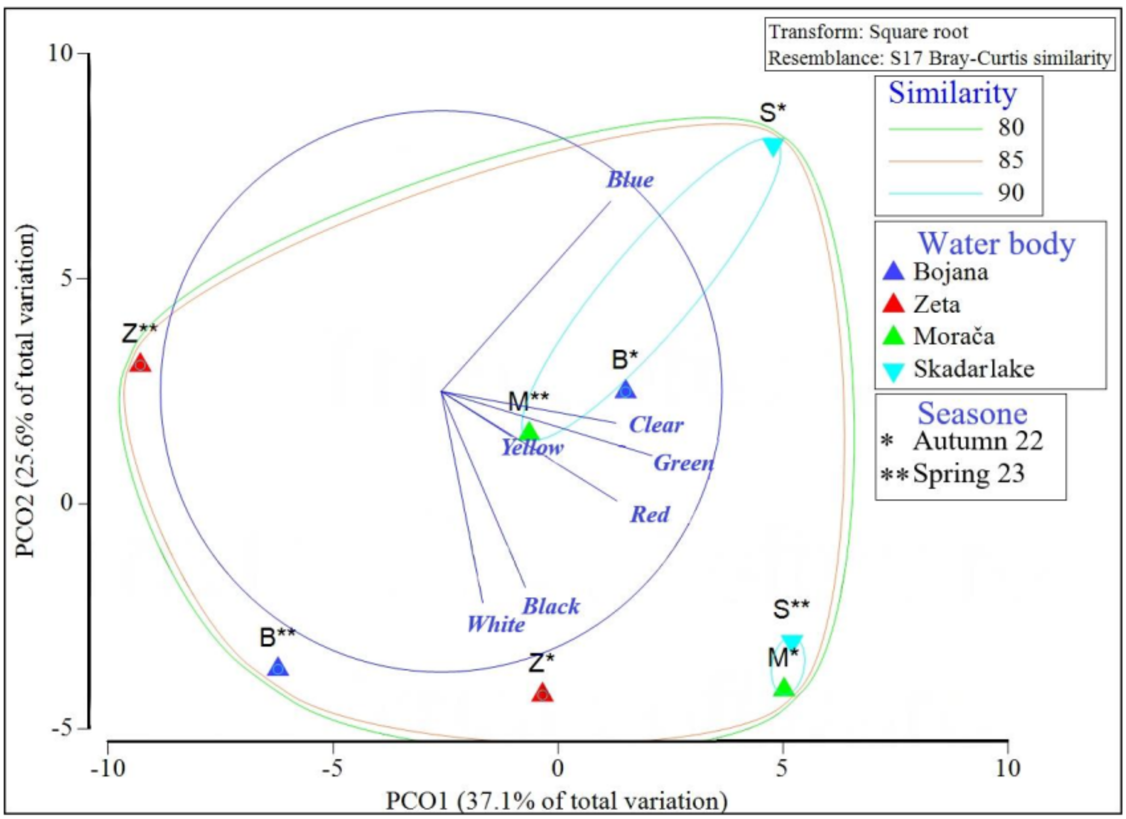


Figure 5.8 PCO and CA analysis of MP color abundance in sediment samples of rivers and lake during the entire research period

CA analysis indicates the presence of five clusters with mutual similarity of 80 %, Figure 5.8. The first cluster includes surface inshore sediment samples of the Zeta river sampled during the spring sampling season where a higher prevalence of clear and blue color was recorded, Figure 5.8. The second cluster includes surface inshore sediment samples of the Bojana river sampled during the spring sampling season, where a similar abundance of blue and red, yellow and green colors and a greater abundance of black and clear colors were recorded, Figure 5.8. The third cluster includes surface inshore sediment samples of the Zeta river sampled during the autumn sampling season, where a higher prevalence of yellow, red and black colors was recorded, Figure 5.8. The fourth cluster includes surface inshore sediment samples of the Morača river sampled during the autumn season and Skadar lake sediment samples sampled during the spring sampling season. The mentioned cluster is characterized by a greater abundance of white and clear colors and a similar abundance of red, blue and green colors, Figure 5.8. The surface inshore sediment samples of the Bojana river and Skadar lake sampled during the autumn season and the Morača river sediment samples sampled during the spring sampling season form the fifth cluster, which is characterized by a greater abundance of blue color and a similar abundance of green and clear colors, Figure 5.8. PERMANOVA, Monte Carlo test and PCO analysis, the sum of the two main components of which is 62.7% of the total variation, indicate that there is no significant statistical correlation of the samples based on the abundance of MP color in relation to the examined water bodies and the sampling season ($p > 0.05$), Figure 5.8.

In river and lake sediments in this study and in sea sediments, fish and mussels from the Montenegrin coast, filaments (fibers), followed by fragments, were the most abundant shape of MP, most often blue, red and clear, medium in size (Bošković et al., 2021, 2022a, 2022b, 2022c, 2023), which confirms that the investigated rivers and lake of the Adriatic basin of Montenegro are one of the important sources of MP on the Montenegrin coast.

5.6 Comparison of the Results of the Visual Identification of Microplastics in the Inshore Sediments of the Studied Rivers and Lake with Available Data from the Literature

During the entire research, in the inshore river sediment samples (Zeta, Morače and Bojane), the most common MP shape types were fibers and fragments, which is in accordance with previous studies that examined the abundance of MP shapes in river sediments (Klein et al., 2015; Cannas et al., 2017; Guerranti et al., 2017; Vermaire et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon-Sanchez et al., 2019; Blair et al., 2019; Gerolin et al., 2020; Constant et al., 2020; Kiss et al., 2021; Matjašič et al., 2022), Table 4.1. Contrary to the above, in the rivers: Elbe in Germany (Scherer et al., 2020) and Saint Laurent in Canada (Crew et al., 2020), the most abundant MP shapes in river sediments were granules, i.e. films in river sediments of Daljao and Huanpu from China (Xu et al., 2020), Table 4.1.

Also, during the entire research, in the inshore sediment samples of Skadar lake, the most common MP shape types were fibers and fragments, which is in accordance with previous studies that examined the abundance of MP shapes in lake sediments (Fischer et al., 2016; Abidli et al., 2017; Turner et al., 2019; Scopetani et al., 2019; Egessa et al., 2019; Felismino et al., 2021), Table 4.2. Granules were not identified in the inshore sediment samples of Skadar lake, while in Ulansuhai lake in China (Qin et al., 2019) and Michigan lake in the USA (Lenaker et al., 2019) granules were the dominant MP shape types, Table 4.2.

Hernandez et al. (2017) state that the sources of fiber in aquatic ecosystems are mainly: wastewater discharges, with an emphasis on wastewater from washing machines; equipment for fishing activities, as well as the textile industry. While fragments in aquatic ecosystems most often originate as a result of the degradation of solid macro- and meso-plastics to plastics of the MP size range. Films in aquatic ecosystems originate as a consequence of the decomposition and degradation of plastic products such as packaging, bags or plastic wraps (Wang et al., 2016). Granules, that are the least represented category of MP shapes in the presented study, mainly come from cleaning agents and cosmetics preparations that reach aquatic ecosystems via wastewater (Wang et al., 2016).

All identified MP particles in rivers and lake inshore sediment samples were in the MP size range (0.1–5 mm). The predominant MP size class in rivers and lake inshore sediment samples was in the range of 0.5-1 mm and 1-3 mm, which is in accordance with literature data for MP identified in river and lake sediments (Klein et al., 2015; Abidli et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon-Sanchez et al., 2019; He et al., 2019; Turner et al., 2019; Qin et al., 2019; Egessa et al., 2019; Scherer et al., 2020; Gerolin et al., 2020; Felismino et al., 2021; Matjašič et al., 2022), Table 4.1 and 4.2. Matjašič et al. (2022) state that the MP particle size affects the possibility of uptake of MP by aquatic organisms, determines the fate of behavior of MP in the aquatic environment, as well as (not) the possibility of removing MP from aquatic ecosystems.

Colored MP particles were identified in all rivers and lake inshore sediment samples in this study, with blue, red, and clear being the most abundant colors. These results are in accordance with previous studies that examined the abundance of MP color in river and lake sediments (Corocran et al., 2015; Vermaire et al., 2017; Abidli et al., 2017; Cannas et al., 2017; Guerranti et al., 2017; Blašković et al., 2018; Rodrigues et al., 2018; Simon-Sanchez et al., 2019; Blair et al., 2019; He et al., 2019; Turner et al., 2019; Yuan et al., 2019; Qin et al., 2019; Lenaker et al., 2019; Scherer et al., 2020; Gerolin et al., 2020; Felismino et al., 2021; Matjašič et al., 2022), Tables 4.1 and 4.2. Abidli et al. (2017) indicate that the blue and clear color of MP may indicate that plastic in aquatic ecosystems originates from the degradation of plastic bottles, foils, bags, wrappers, etc., therefore it may indicate that the identified MP originates from packaging waste. Also, colored particles of MP are very attractive to aquatic organisms, whereby aquatic organisms very often ingest them replacing them with food (Browne et al., 2011).

6. CHEMICAL IDENTIFICATION OF MICROPLASTICS IN RIVERS AND LAKE SEDIMENT

Out of a total of 642 visually identified MP particles during the entire research, 28 % were chemically identified, of which 13.2 % by ATR FTIR and 14.8 % by μFTIR. The MP particles selected for chemical analysis are particles of different shape, color and size from each individual sample. Twelve MP particles analyzed by FTIR were not polymer structures (organic and inorganic components) that were not included or shown in the results, and the results were recalculated relative to them.

In samples of surface inshore sediments of rivers and lake, the following polymers were chemically identified by FTIR: polypropylene (PP 28.4 %), polyethylene (PE 28.4 %), polyethylene terephthalate (PET 19.1 %), polyamide (PA 13.6 %), polystyrene (PS 4.7 %), polyvinyl chloride (PVC 3.1 %), acrylate copolymer (Acrylat cop. 1.2 %), polyvinyl alcohol (PVA 0.8 %) and polytetrafluoroethylene (PTFE 0.8 %).

Figure 6.1 shows the IR spectra of the identified types of polymers in the studied samples of inshore sediments of rivers and lake.

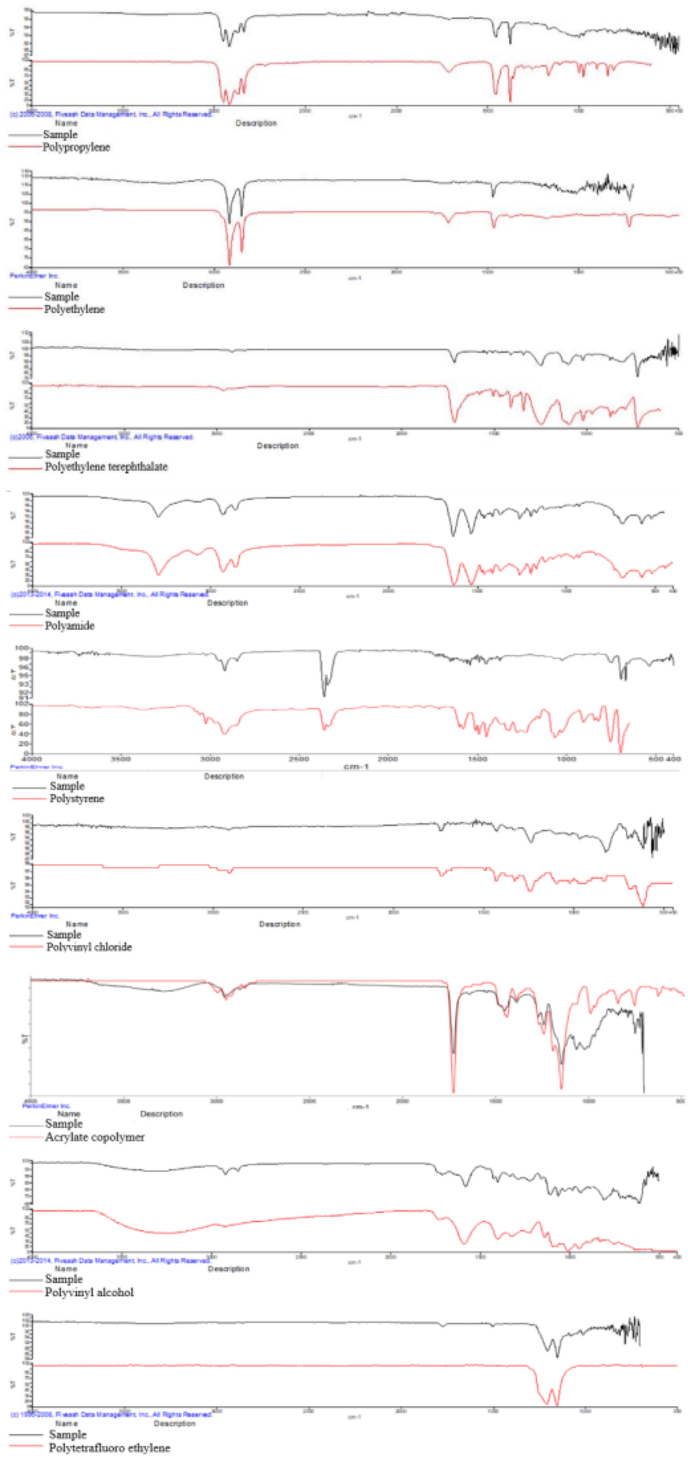


Figure 6.1 Presentation of examples of IR spectra of identified types of polymers in examined sediment samples of rivers and lake

6.1 Zeta

The percentage abundance of MP polymer types in the surface inshore sediment of the Zeta river in relation to the sampling season and during the entire research period is shown in Figure 6.2.

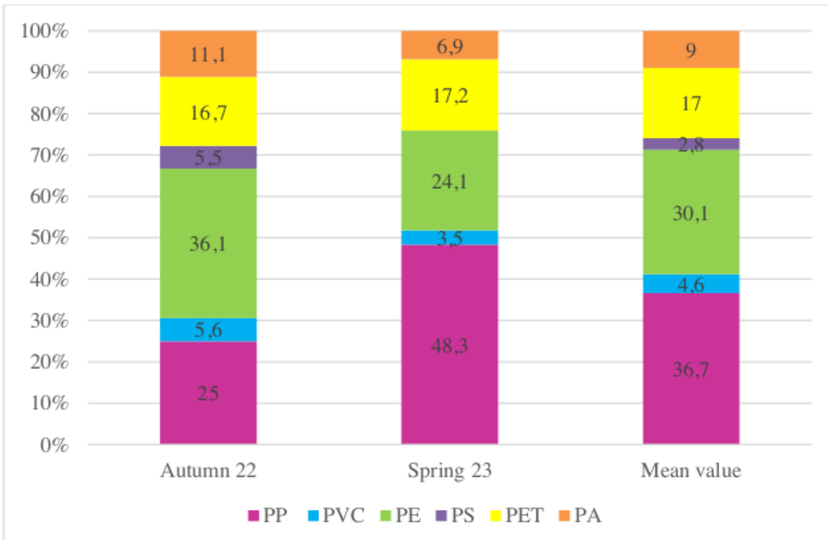


Figure 6.2 Percentage abundance of MP polymer types in sediment of the Zeta river based the sampling season and during the entire research period

The abundance of polymer types in the inshore sediment of the Zeta river during the entire study was in the following sequence: PP (36.7 %) > PE (30.1 %) > PET (17 %) > PA (9 %) > PVC (4.6 %) > PS (2.8 %), Figure 6.2. The abundance of polymer types in the inshore sediment of the Zeta river differed in relation to the sampling season, so during the autumn season it ranged: PE > PP > PET > PA > PS > PVC, i.e.: PP > PE > PET > PA > PVC during the spring sampling period, Figure 6.2. During the autumn season, the dominant polymer type in the inshore sediment of the Zeta river was PE, and during the spring season, PP, while PS was identified only in the inshore sediment of the Zeta river during the autumn sampling season, Figure 6.2.

6.2 Morača

The percentage abundance of MP polymer types in the surface inshore sediment of the Morača river in relation to the sampling season and during the entire research period is shown in Figure 6.3.

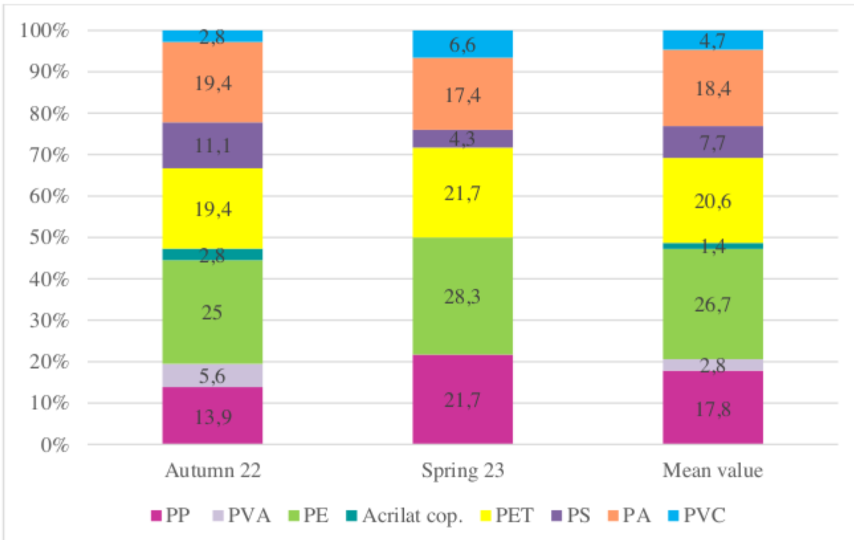


Figure 6.3 Percentage abundance of MP polymer types in sediment of the Morača river based the sampling season and during the entire research period

In the inshore sediment of the Morača river during the entire research, the abundance of polymer types was in the following sequence: PE (26.7 %) > PET (20.6 %) > PA (18.4 %) > PP (17.8 %) > PS (7.7 %) > PVC (4.7 %) > PVA (2.8 %) > Acrylic cop. (1.4 %), Figure 6.3. The abundance of polymer types in the inshore sediment of the Morača river differed in relation to the sampling season, so during the autumn season it moved in the following sequence: PE > PA > PET > PP > PS > PVA > Acrilat cop. > PVC, ie: PE > PP > PET > PA > PVC > PS during the spring sampling period, Figure 6.3. During both sampling seasons, the dominant type of polymer in the inshore sediment of the Morača river was PE, while Acrilat cop. and PVA were identified only in the inshore sediment of the Morača river during the autumn sampling season, Figure 6.3.

6.3 Bojana

The percentage abundance of MP polymer types in the surface inshore sediment of the Bojana river in relation to the sampling season and during the entire research period is shown in Figure 6.4.

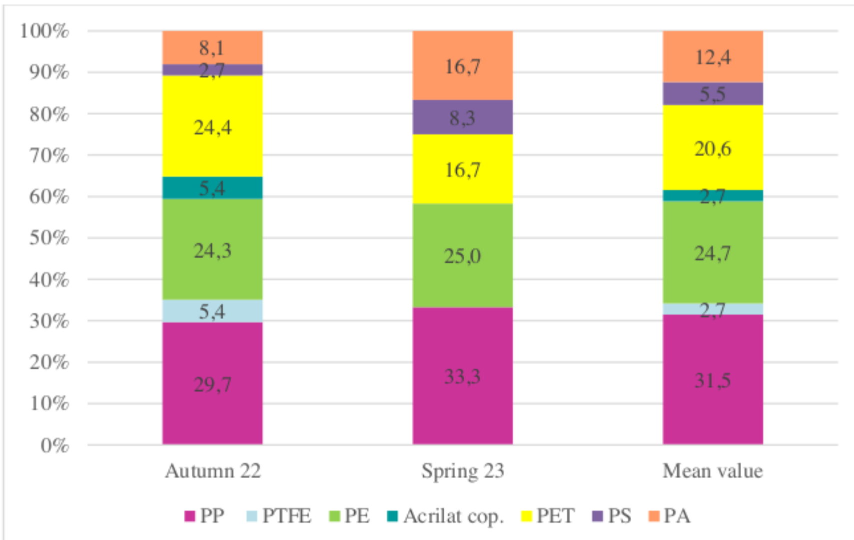


Figure 6.4 Percentage abundance of MP polymer types in sediment of the Bojana river based the sampling season and during the entire research period

During the entire research, the abundance of polymer types in the inshore sediment of the Bojana river moved in the following sequence: PP (31.5 %) > PE (24.7 %) > PET (20.6 %) > PA (12.4 %) > PS (5.5 %) > PTFE (2.7 %) > Acrylic cop. (2.7 %), Figure 6.4. The abundance of polymer types in the inshore sediment of the Bojana river differed in relation to the sampling season, so during the autumn season it moved in the following sequence: PP > PET > PE > PA > Acrylic cop. > PTFE > PS, ie: PP > PE > PET > PA > PS during the spring sampling period, Figure 6.4. During both sampling seasons, the dominant polymer type in the inshore sediment of the Bojana river was PP, while Acrilat cop. and PTFE were identified only in the inshore sediment of the Bojana river during the autumn sampling season, Figure 6.4.

6.4 Skadar lake

The percentage abundance of MP polymer types in the surface inshore sediment of Skadar lake in relation to the sampling season and during the entire research period is shown in Figure 6.5.

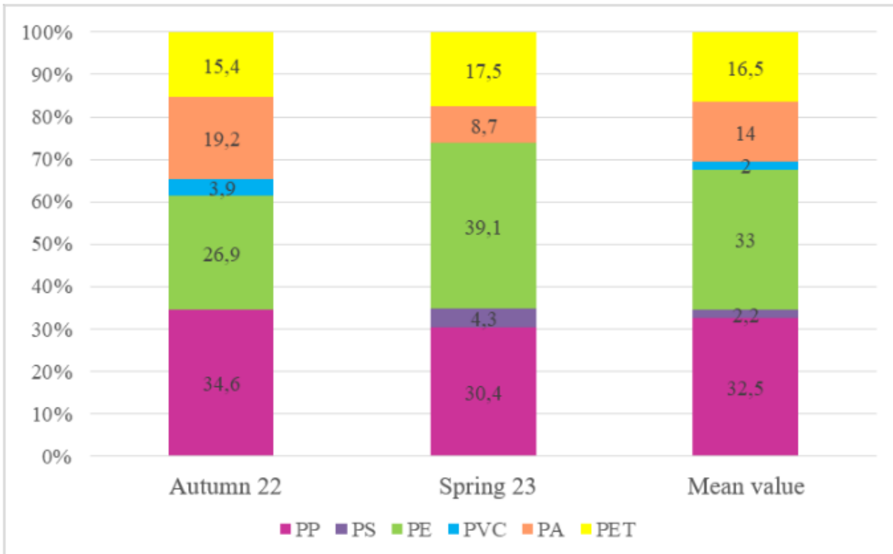


Figure 6.5 Percentage abundance of MP polymer types in sediment of the Skadar lake based the sampling season and during the entire research period

The abundance of polymer types in the inshore sediment of Skadar lake during the entire research was in the following order: PE (33 %) > PP (32.5 %) > PET (16.7 %) > PA (14 %) > PS (2.2 %) > PVC (2 %), Figure 6.5. The abundance of polymer types in the inshore sediment of Skadar lake differed in relation to the sampling season, so during the autumn season it moved in the following sequence: PP > PE > PA > PET > PVC, i.e.: PE > PP > PET > PA > PS during the spring sampling period, Figure 6.5. During the autumn season, the dominant polymer type in the inshore sediment of Skadar lake was PP, and during the spring season, PE, while PVC was identified only during the autumn season, and PS only during the spring sampling season in the inshore sediment of Skadar lake, Figure 6.5.

6.5 Comparative Analyzes of the Results of the Chemical Identification of Microplastics

PCO and CA analysis of MP polymer type abundance in surface inshore samples of rivers and lake sediments during the entire research period is shown in Figure 6.6.

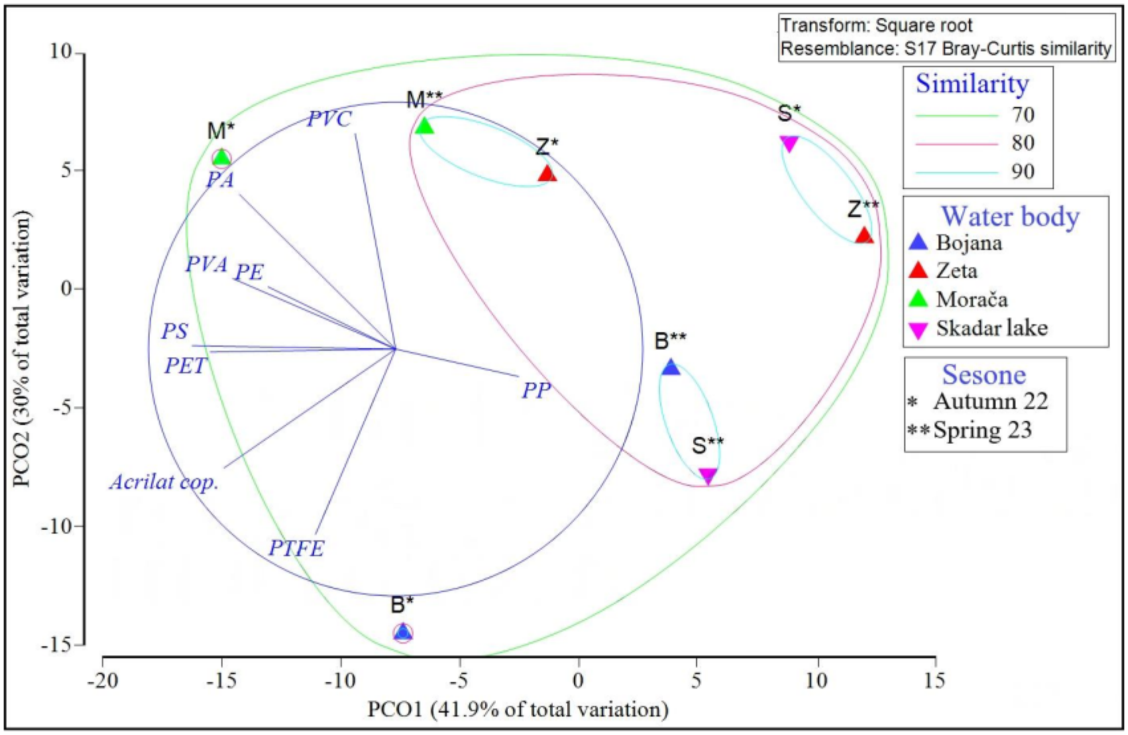


Figure 6.6 PCO and CA analysis of MP polymer type abundance in sediment samples of rivers and lake during the entire research period

Figure 6.6 shows three clusters with mutual similarity of 70 %, Figure 6.6. The first cluster includes the surface inshore samples of the sediment of the Morača river during the autumn sampling season, where only the abundance of PVA was recorded, while in the second cluster, which includes the surface inshore samples of the sediment of the Bojana river during the autumn sampling season, the only abundance of PTFE was recorded, which is why the above two clusters appear independently, Figure 6.6. What connects the first and second clusters is the abundance of Acrilat cop., Figure 6.6. The third cluster consists of three subclusters. The first subcluster consists of surface inshore sediment samples of the Zeta and Morača sampled during the autumn and spring sampling seasons, and is characterized by a higher prevalence of PVC and PA, Figure 6.6. The second subcluster consists of surface inshore sediment samples of Skadar lake and the Zeta river sampled during the autumn and spring sampling seasons, and is characterized by a greater abundance of PP and the absence of PS, Figure 6.6. The third subcluster consists of surface inshore sediment samples of Skadar lake and the Bojana river sampled during the spring sampling season, and is characterized by a greater abundance of PP, an equal abundance of PET and the absence of PVC, Figure 6.6. PERMANOVA, Monte Carlo test and PCO analysis, whose sum of the two main components is 61. 9% of the total variation, indicate that there is no significant statistical correlation of the samples based on the abundance of MP polymer types in relation to the examined water bodies and the sampling season ($p > 0.05$), Figure 6.6.

A comparative analysis of the abundance of MP polymer types in the investigated inland waters of the Adriatic basin in this study, with the abundance of MP polymer types on the Montenegrin coast (Bošković et al., 2021, 2022a, 2022b, 2022c, 2023) indicates that in both investigated areas, the most represented polymer types are PP and PE. The above indicates, once again, that the examined inland waters of the Adriatic basin in this study are significant sources of MP on the Montenegrin coast.

6.6 Comparison of the Results of the Chemical Identification of Microplastics in the Inshore Sediments of the Studied Rivers and Lake with Available Data from the Literature

During the entire research, in the inshore river sediment samples (Zeta, Morače and Bojana), the most abundant MP polymer types were PE, PP, followed by PET and PA, which is in accordance with previous studies that examined the abundance of MP polymer types in river sediments (Klein et al., 2015; Rodrigues et al., 2018; He et al., 2019; Atwood et al., 2019; Simon-Sanchez et al., 2019; Scherer et al., 2020; Constant et al., 2020; Xu et al., 2020; Matjašič et al., 2022), Table 4.1.

Also, during the entire research, in the inshore sediment samples of Skadar lake, the most abundant MP polymer types were PE, PP, which is in accordance with the data from the literature that dealt with the examination of the abundance of MP polymer types in lake sediments (Corocran et al., 2015; Yuan et al., 2019; Qin et al., 2019; Lenaker et al., 2019; Egessa et al., 2019; Felismino et al., 2021), Table 4.2.

Yuan et al. (2019) indicate that the results of the chemical identification of MP polymers can help to identify a trace of the original form of plastic residues in the environment. PE and PP have been reported in the literature as the two types of polymers with the largest global volume of production and use, large distribution, and therefore a higher probability of becoming plastic waste in freshwater, estuarine and marine ecosystems (Plastics Europe, 2021). The higher prevalence of PE and PP in this study is consistent with their global production and widespread use. PE and PP are used to make: reusable bags, disposable utensils, food packaging, cosmetic bottles, agricultural films, fishing nets and ropes, plastic container caps, bottled water caps, toys, household utensils and consumer bags, textile floor coverings, carpets, mats, pipes, sports clothes, etc. (Plastics Europe, 2021). PET has high strength, good elasticity and strong heat resistance and thermoplasticity, which makes it a useful material in: textile industry, as a packaging material, construction and automotive industry (Jiang et al., 2019; Xu et al., 2020). PS is widely used in packaging and for the production of disposable items, for the production of microbeads in personal care products and cosmetics, and in construction (Jiang et al., 2019; Ding et al., 2021). PA is used in the production of various fabrics and bags, fishing equipment (nets, ropes), bags, wrappers, foils, packaging, etc. (Plastics Europe, 2021). PVC is widely used in various industries and products in construction, electronics, furniture, pipes, windows and doors, insulators, toys, clothing, packaging, and even in healthcare (Egessa et al., 2019). PVC can release harmful plasticizers, antiaging agents, and other elements, and is also a major source of dioxins, which are highly toxic to aquatic organisms and ultimately affect humans in the food chain (Jiang et al., 2018). Acrylate cop. has found wide application in the cosmetic industry, it is used in various body and hair care products (Yayayürük, 2017).

The presented results of this study provide precise information about the abundance of different types of MP polymers in the analyzed river and lake sediments and potential sources, which is necessary for taking preventive measures, monitoring and future research.

7. ECOLOGICAL ASSESSMENT OF THE RISKS FROM MICROPLASTICS

The hazard classification of identified polymers in inshore surface sediments of rivers and lake throughout the study is shown in Table 7.1. The potential hazards of the identified polymers to human health, aquatic organisms and the environment have been checked by the European Chemical Agency - ECHA (www.echa.europa.eu/home).

Table 7.1 Hazard classification of identified polymers in sediments of rivers and lake throughout the study (www.echa.europa.eu/home)

Identified polymers	Total abundance in rivers and lakes	EC / list number	Hazard classification and labeling by ECHA and CLP
PP	28.4 %	618-352-4	No hazards are classified.
PE	28.4 %	618-339-3	No hazards are classified.
PET	19.1 %	607-507-1	No hazards are classified.
PA	13.6 %	943-936-3	**Dangerous!** This substance causes severe skin burns and eye damage, is toxic to aquatic life with long-term effects, is harmful if swallowed, is harmful if inhaled, may cause an allergic skin reaction, and may cause respiratory irritation.
PS	4.7 %	935-499-2	**Warning!** This substance is harmful to aquatic life with long-lasting effects.
PVC	3.1 %	208-750-2	**Dangerous!** This substance is highly flammable, harmful by inhalation and harmful to aquatic life with long-term effects.
Acrilat cop.	1.2%	607-559-5	**Warning!** This substance causes serious eye irritation, may cause long-term adverse effects in aquatic animals and causes skin irritation.
PTFE	0.8 %	618-337-2	No hazards are classified.
PVA	0.8 %	618-340-9	**Warning!** This substance can cause organ damage.

Nine types of polymers were identified in surface inshore sediments of rivers and lake, of which PP, PE, PET and PTFE are classified by ECHA as non-hazardous, PA and PVC as dangerous, and PS, Acrylat cop. and PVA with warning signals (Table 7.1). Although the most abundant polymers in this study (PP, PE and PET), which make up ~ 80% of the total abundance of the identified polymers, are classified as non-hazardous according to ECHA, the fact that should not be ignored is that the plastics industry uses a wide range of additives with different properties that can be released from plastics during its life cycle which leads to human and environmental exposure (Hahladakis et al., 2018). The results presented in this study provide data on the impact of the chemical composition of pure "virgin" polymers identified in rivers and lake sediments on aquatic organisms, the environment and human health, which may provide important information for future research.

The results of PLI have classified rivers and lake sediments at each investigated location separately, as well as in rivers and lake during the entire research period in total, into category I (Table 3.2). According to PLI values, the sediments of the Zeta, Morača and Bojana rivers and Skadar lake are slightly contaminated with MP. Contrary to the mentioned PLI results, by comparison with the available literature data on the abundance of MP in freshwater ecosystems, the examined sediments in this study are moderately to highly polluted with MP, depending on the investigated location. Similar results were obtained in studies dealing with the assessment of PLI values in freshwater ecosystems (Wang et al., 2021; Warrier et al., 2022; Amrutha et al., 2023; Ephsy and Raja, 2023). Overall, the lower PLI values (< 10) found in the studied rivers and lake in this study are the consequence of relatively high MP background values. The selection of an appropriate MP background value is very important for understanding the estimation of PLI (Ranjani et al., 2021). Authors Wang et al. (2021), Ranjani et al. (2021) and Gurumoorthi and Lewis (2023) state that a key task for future research is to set a reference MP background value for MP pollution load estimation in order to obtain more accurate data. Based on the available studies dealing with the estimation of PLI for MP (Wang et al., 2021; Ranjani et al., 2021; Gurumoorthi and Lewis, 2023), in this study, the lowest abundance of MP discovered in sediment samples of rivers and lake was taken into account as a background value.

Based on the PHI value, the total risk of MP pollution in the sediment of the Zeta river is classified as danger level IV (100-1000), while the total risks of MP pollution in the sediments of the Morača and Bojana rivers and Skadar lake are classified as danger level V (>1000) (Table 3.2). PHI values indicate a serious trend of MP pollution. High PHI values are the result of high abundance of MP with high hazard ratings, such as Acrylat cop., PA and PS (Table 3.2). Similar observations were made by Amrutha et al. (2023), Kasamesiri et al. (2023) and Ranjani et al. (2021) in their studies.

The results indicate that the increased risk of MP pollution depends both on the level of MP in the environment and on the level of abundance of harmful MP polymers (Xu et al., 2018). The combined use of PLI and PHI in this study provided a preliminary quantitative measure of the ecological risks caused by MP pollution in the studied rivers and lake.

8. THE PROPOSED MODELS FOR SOLVING THE PROBLEM OF PLASTICS/MICROPLASTICS IN MONTENEGRO

Since MP is a global problem, in order to manage and solve this problem, it is necessary to combine forces through the joint action of the government, local authorities, companies, the non-governmental sector, the scientific community and individuals.

As a future member of the European Union, Montenegro harmonizes its laws and regulations with those of the European Union. However, the MP is not yet subject to the regulations of Montenegro. The analysis of the Montenegrin legislation determined that the laws and other legal acts MP do not classify it as a pollutant, therefore monitoring the presence of MP in the environment is not mandatory. As an ecological country, Montenegro must recognize this ecological problem and include it in the existing legal framework.

Montenegrin document in which one subchapter is dedicated to the problem of plastics/MP is the "*Marine Environment Monitoring Program of Montenegro (2022)*". This document describes recommendations for monitoring floating waste by visual observation and sampling MP in the water column in the area of the Boka Kotorska Bay. On the Montenegrin coast, the abundance of macroplastics in seawater and the gastrointestinal tract of certain fish species was determined through several different projects (Zeri et al., 2018; Anastasopoulou et al., 2018). The literature review showed that there are currently three studies on the abundance of MP in the surface sediment of the Montenegrin coast (Bošković et al., 2021, 2022a, 2022b, 2022c), one study on the abundance of MP in commercially important fish species from the Montenegrin coast (Bošković et al., 2022b), and one study on the abundance of MP in mussels (Bošković et al., 2023), while there are no studies, until this study, on the abundance of (meso and/or micro) plastics in the freshwater ecosystems of Montenegro. The aforementioned studies indicate that the aquatic ecosystems of Montenegro are polluted by MP of different polymer structures, which requires needs and actions to solve this problem.

One of the proposed models and possible solutions is the establishment of a Center for testing plastics/MP in biotic and abiotic factors of the environment. The activities of the Center would be based on:

– cooperation with the Government of Montenegro, ministries, agencies and the non-governmental sector;

- adopting policies related to plastics/MP;
- compliance with directives and legal regulations of the European Union regarding the management of this type of waste;
- establishing a regular environmental monitoring program and preventing further plastic/MP pollution;
- raising awareness about the existence of MP, its impact on the environment, ecosystems, animals, humans and its life cycle;
- labeling of products that contain MP;
- creating a catalog of plastics that are imported and produced in Montenegro;
- developing methods for identification of MP;
- finding solutions to reduce plastic waste (prevention);
- proposal and development of methods for reuse and recycling of plastic products;
- development of a plastic waste management and disposal strategy at the level of Montenegro;
- development of wastewater management and treatment strategy at the level of Montenegro;
- reduce (ban) the use of single-use plastic packaging;
- strengthen the use of natural and/or biodegradable materials;
- strengthen and organize public cleaning actions;
- carry out education of the population through workshops, seminars, conferences, lectures, public debates in order to raise awareness about the global problem of MP, as well as possible solutions and alternatives.

A similar model was proposed by Teofilović et al. (2021) for the Republic of Serbia. The data obtained from the work of the Center for testing plastics/MP would be of great importance both for the citizens of Montenegro and for Europe, but also for the entire Planet, because plastics/MP in the environment is a global problem. Due to its numerous advantages, it is impossible to completely ban plastics or put it out of use, but the Government of Montenegro should first recognize this environmental problem and include it in the existing legal framework.

The second proposed model in plastics/MP management would be based on the following:

- **Supporting research**

  It is necessary for the state/government to allocate funds to support research, development of incentives and subsidies in the area of plastic/MP management in order to find safer consumer products for the environment and human health during their life cycle. The above can also help in the development of new technologies and materials that are sustainable, cost-effective and have a reduced negative impact on the environment and human health.

- **Establishing regulations**

  Policymakers need to establish regulations and standards that limit the use of certain types of plastic. Through education, it is necessary to present to the population the advantages of switching from traditional to alternative plastic (bio-based plastic), as well as find ways and promote the reuse and recycling of plastic.

- **Establishing collaboration**

  Encouraging collaboration between industry, academia and research institutions is key in the plastics/MP management sector. Joint efforts can stimulate innovation, accelerate the development and commercialization of alternatives. Policy makers can encourage individuals (population) to contribute to reducing plastic pollution by promoting responsible consumption and EU waste management practices.

- **Development of infrastructure**

  The development of adequate infrastructure such as recycling plants, waste collection systems and reuse on a large-scale are very important. The development of adequate infrastructure can support the goals of encouraging extended producer responsibility, therefore making producers and consumers of plastic products more responsible during all phases of the life cycle of plastic products, with an emphasis on the collection, recycling and reuse of plastics.

- **Economic incentives**

  Economic incentives can significantly influence producer and consumer behavior by promoting sustainable practices which may include deposit refund systems for plastic packaging and financial support for recycling. Economic incentives can encourage research and development in the field of plastic waste management, promoting the invention of new technologies, materials and processes that reduce the generation of plastic waste, improve recycling rates and promote circular economy models.

A model of the circular economy as a manner of solving the problem of plastic/MP pollution requires action of interested parties at many different levels, including producers of raw materials for the production of plastics, producers of plastics, companies that produce or sell consumers goods, retailers, consumers, waste managers, authorities for waste management and recyclers of plastics (Ryberg et al., 2018). Prieto-Sandova et al. (2018) indicated that circular economy requires innovation in the ways in which industries produce, consumers use and policy makers make laws. The circular economy promotes the design of systems of closed loop that prevent that plastics become waste. Using plastic products through sharing, repairing and reusing approaches the ideal of the circular economy. One of the models of the circular management of plastics is based on the following principles: 1) product as a service ("pay after use"); 2) circular supplies (waste from one company becomes raw material for another); 3) extension of the life of products (makes products permanent, repairable, upgradable) and 4) platforms for sharing (Wagner, 2021). Based on the aforementioned, the implementation of the model of the circular economy is recommended as an effective way for solving the problem of plastic/MP pollution.

Although we will have to face many technological, managerial and social challenges on our way to solve plastic/MP pollution in Montenegro, there are some conditions that will facilitate the journey. This includes strong evidence from the natural and social sciences about the abundance of MP in the Montenegrin environment, the effectiveness of different solutions, a broad willingness to solve the problem and acceptance of shared responsibility. It is important to emphasize that all participants in the system of plastics are responsible for the generation, management and solving the problem of plastic pollution.

CONCLUSION

This study provides significant data on: abundance, distribution, sources of MP in rivers and lake sediments, ecological assessment of the examined water bodies, visual and chemical characteristics of MP, potential impacts of MP on aquatic organisms, the environment and human health, assessment of the examined water bodies of the Adriatic basin as a source of MP on the Montenegrin coast, as well as suggestions for solutions and future studies.

- The MP abundance in the inshore sediments of the examined water bodies of the Adriatic basin was in the following sequence: Bojana (180 ± 53.5 MP/kg dry sediment) > Morača (169 ± 113 MP/kg dry sediment) > Skadar lake (153.4 ± 42.7 MP/kg dry sediment) > Zeta (145 ± 110 MP/kg dry sediment). The total mean abundance of MP during the entire study in the studied rivers and lake of the Adriatic basin was 160.5 ± 83.3 MP/kg of dry sediment. CA analysis, PERMANOVA and Monte Carlo test indicate that there is no significant statistical correlation in the level of MP abundance in relation to the examined water bodies, locations and sampling season ($p > 0.05$). Based on the comparison with literature data, the studied rivers and lake are moderately polluted by MP.
- The identified factors that can be linked to the sources, abundance and distribution of MP in inshore sediments of rivers and lake of the Adriatic basin are: hydrodynamic and ecological conditions; population density, tourist fishing and agricultural activities, wastewater discharge, inadequate solid waste management, as well as cross-border pollution.
- The MP abundance of shapes in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) followed the following sequence: fibers > fragments > films > granules. In the examined sediment samples, fibers, followed by fragments, were the most abundant MP shape, while granules were not identified in the sediments of Skadar lake. PCO analysis, PERMANOVA and Monte Carlo test indicate that there is no significant statistical correlation in the level of MP abundance of shape type in relation to the examined water bodies and the sampling season ($p > 0.05$).
- The MP abundance of size in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) was in the following

sequence: 0.5-1 mm > 1-3 mm > 3-5 mm > 0.1-0.5 mm. The results indicate that MP of the medium size category is the most abundant in the investigated sediment samples. PCO analysis, PERMANOVA and Monte Carlo test show a statistical correlation of MP size abundance in relation to the sampling season ($p < 0.05$), while no statistical correlation of MP size abundance is observed in relation to the examined water bodies ($p > 0.05$).

- The MP abundance of color in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) was in the following order: blue > clear > red > black > yellow > green > white. Blue, clear and red are the most represented color categories in the examined sediment samples. PCO analysis, PERMANOVA and Monte Carlo test indicate that there is no significant statistical correlation in the level of MP color abundance in relation to the examined water bodies and the sampling season ($p > 0.05$).
- The MP abundance of polymers in the inshore sediments of all examined water bodies of the Adriatic basin (rivers: Zeta, Morača, Bojana and Skadar lake) was in the following sequence: PP > PE > PET > PA > PS > PVC > Acrylic cop. > PVA > PTFE. PP was the most abundant type of polymer in the sediments of the Zeta and Bojana rivers, while PE was the most abundant type of polymer in the sediments of the Morača river and Skadar lake. PCO analysis, PERMANOVA and Monte Carlo test indicate that there is no significant statistical correlation in the level of abundance of MP polymer types in relation to the examined water bodies and the sampling season ($p > 0.05$).
- Potential hazards of identified polymers in surface inshore sediments of rivers and lake to human health, aquatic organisms and the environment checked by ECHA whose classification is based on the chemical composition of pure "virgin" polymers classified the polymers as follows: PP, PE, PET and PTFE harmless, PA and PVC dangerous, and PS, Acrylat cop. and PVA with warning signals. The most abundant polymers in this study (PP, PE and PET) are classified as non-hazardous according to ECHA, however, we should not ignore the fact that the plastics industry uses a wide range of additives with different properties that can be released from the plastic during its life cycle, leading to human and environmental exposure. Potential ecological risks from MP in the studied freshwater ecosystems of Montenegro are insignificant in relation to PLI values, i.e. extremely high in relation to PHI values.

- The results indicate that the investigated freshwater ecosystems are not only the pathways of MP emission from the land to the seas and oceans, but also secondary sources and reservoirs of previously accumulated MP. This study indicates that the identified visual and chemical characteristics of MP do not differ between freshwater ecosystems in this study and marine ecosystems in previously examined studies in Montenegro (Bošković et al., 2021, 2022a, 2022b, 2022c, 2023), so it can be concluded that the inflow of inland waters from the Adriatic basin contributes to the increase in the abundance of MP on the Montenegrin coast. These findings highlight the urgency of further monitoring freshwater ecosystems and identifying point sources to mitigate MP contamination of aquatic ecosystems in the near future.
- The results of this study indicate that the identified MP originates from wastewater and fragmentation of larger plastic remains, which indicates a large use of plastic and its inadequate disposal by the population of Montenegro. Therefore, control of plastic/MP at source is an option that needs serious attention. Legislation and decisions at the local and national level regarding the reduction of plastic use, especially single-use plastics and plastic packaging, are key in reducing and solving the problem of plastic/MP pollution. The Government of Montenegro through coordinated efforts should optimize and improve the processes and management of WWTP and waste management at the level of Montenegro, as soon as possible.

This study provides the first information about the presence, sources and ecological risk of MP pollution in the examined freshwater ecosystems of Montenegro. Further studies on the temporal variations of MP pollution and the ecological risk of MP are needed in order to improve knowledge about the fate, transport and impacts of MP on the environment and human health. Also, constant monitoring of the impact and fate of MP in freshwater ecosystems is suggested, as well as an assessment of potential impacts on humans that arise from the consumption of fish products.

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