

DERELICT FISHING GEAR MANAGEMENT SYSTEM IN THE ADRIATIC REGION (DEFISHGEAR)



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Pilot assessment on microplastic in rivers

Report for WP5

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Derelict Fishing Gear management system in the Adriatic Region

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Acronyms

EOX - Extractable organic halides

NY - Nylon

PA - Polyamide

PAH - Polycyclic aromatic hydrocarbons

PCB - Polychlorinated biphenyls

PE - Polyethylene

PET - Polyethylene terephthalate

PP - Polypropylene

PS - Polystyrene

Summary

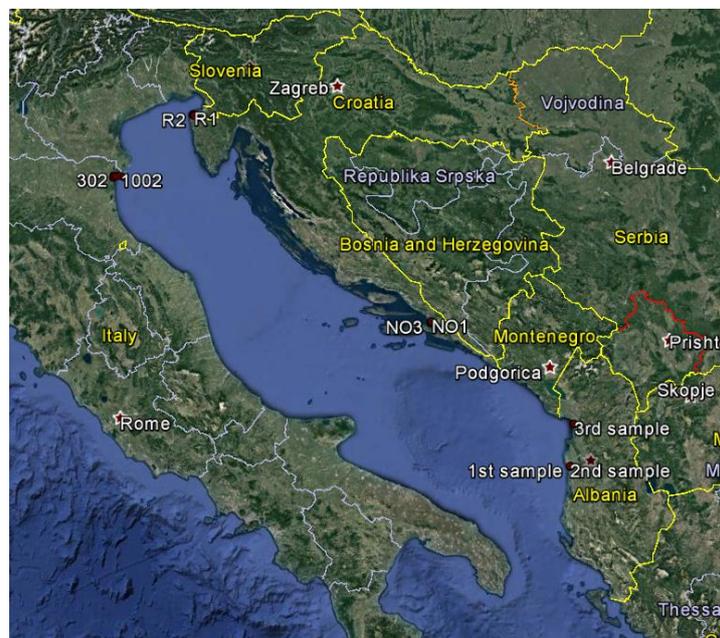
This report summarises findings of part of the work carried by work package 5 (WP5) of the project DeFishGear, referred to the monitoring and assessment of microplastic pollution (plastic particles <5 mm) in the rivers that discharge into the Adriatic Sea. The report is a result of coordinated actions in frame of the DeFishGear project toward sharing knowledge on analysing microplastic pollution, which included application of the joint monitoring methodology in the Adriatic region aiming to determine microplastic types and quantities in coastal and marine departments.

The main objectives of the output “Pilot assessment on microplastic in rivers” were:

- The use of harmonized sampling and sample analysis methodology prepared for the sea surface samples that is usable for all Adriatic region.
- Estimation of microplastic concentration on the river outflows that discharges into the Adriatic Sea.

Methodology

The methodology for sampling and sample analysis of river outflow samples was in all steps the same as for the sea surface sampling and is described in the document “Recommendation on regional approach to monitoring and assessment of microplastic in the marine environment”, from Manca Kovač Viršek, Andreja Palatinus, Helen Kaberi, Catherine Tsangaris, Cristina Mazziotti (December 2015) in the Chapter 2: Sea surface sampling and microplastic separation. In DeFishGear project five river outflows were sampled for microplastic analysis, the Po River outflow in Italy, Dragonja River outflow in Slovenia, Neretva River outflow in Croatia, and rivers Drin and Lumi Durrësit in Albania in the years from 2014 to 2016 (Figure 1).



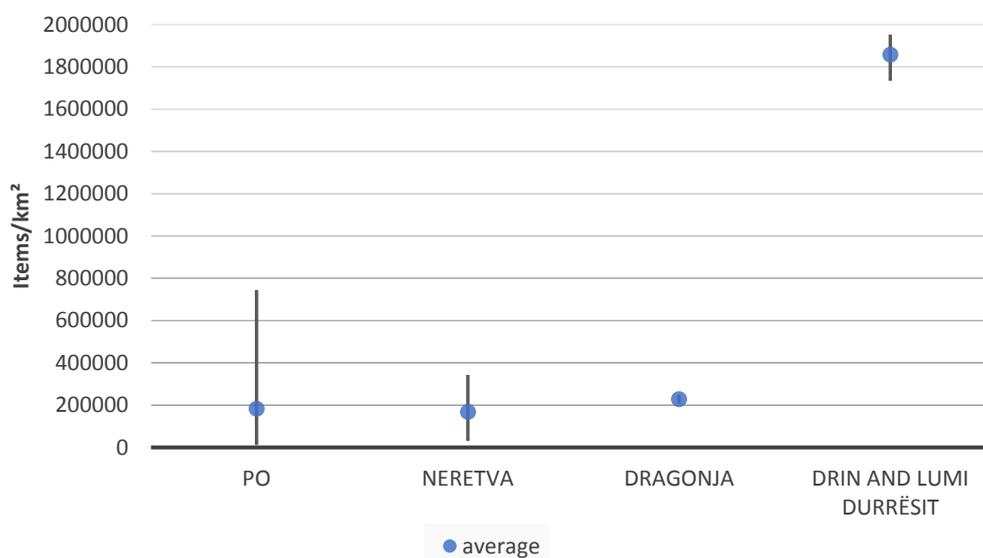
Map of sampling transects on rivers Po in Italy (labels: 302, 1002), Dragonja in Slovenia (labels: R1, R2), Neretva in Croatia (labels: NO1, NO3), and Drin and Lumi Durrësit in Albania (labels: 1st, 2nd and 3rd sample) in years from 2014 to 2016. Photo: Google Earth; Uroš Robič, IWRS.

Derelict Fishing Gear management system in the Adriatic Region

Microplastic separation from the river outflow samples was done by the use of stereomicroscopes. Microplastic particles were counted, weighted, categorized into 6 categories (fragments, filaments, foams, granule, pellets and other) and analysed for their chemical structure. At the end estimation of density of microplastic pollution for each country and all Adriatic region was calculated.

Results

The comparison among Po, Neretva and Dragonja river outflows show the similar average concentrations of microplastic, but the microplastic concentration on the Drin and Lumi Durrësit was almost 10 times higher than in the other three rivers. Therefore Albanian rivers had the highest average concentration (1,857,346 Items/km²) and Neretva River had the lowest concentration (168,600 Items/km²). The concentration range was the largest for the Po River (11,000 – 744,000 Items/km²) and the smallest for the Dragonja River (197,000 – 226,000 Items/km²).



Average number of microplastic particles per km² for all river outflow samplings on rivers Po in Italy, Dragonja in Slovenia, Neretva in Croatia, and Drin and Lumi Durrësit in Albania in years from 2014 to 2016.

Among microplastic categories, the number of particles in category of fragments was the highest (>50% of all founded particles). Fragments were the most prevalent particles in the Po and Neretva river outflow, while in the Dragonja River and Albanian rivers, filaments were the most numerous group of particles. Very interesting results is, that among microplastic categories, pellets were not found in any sample.

Results of chemical analysis of particles were similar for all measured samples, with polyethylene as the most abundant material in Slovenia, followed by polypropylene and polyamide (nylon) in Italy.

Conclusions

- The comparison among Po, Neretva and Dragonja river outflows showed similar average concentrations of microplastics with a range from 168,600 to 228,046 Items/km², but the concentration of particles in Albanian rivers was quite larger than in the other three rivers (1,857,346 Items/km²).
- The most prevalent category of particles were fragments in the Po and Neretva river outflows, and filaments in Dragonja, Drin and Lumi Durrësit river outflows. Interestingly, no pellets were found.
- The most abundant plastic material among microplastic particles sampled from river outflows of the Adriatic Sea was polyamide (nylon) in Italy and polyethylene in Slovenia.
- In order to achieve better results, the use of blank samples at all times during the sample preparation and sample analysis is advised to consider the airborne contamination of fibers. Also the use of clean room or laminar air flow cabinet are welcomed. The use of 30% hydrogen peroxide is recommended, but without heating above 30°C.
- This research, conducted in the frame of DeFishGear project, present the first valuable data on the overall state of the contamination with microplastic in years from 2014 to 2016 on Po, Dragonja, Neretva, Drin and Lumi Durrësit river outflows that discharges into the Adriatic Sea.

Table of contents

Acronyms	iv
Summary	v
Methodology.....	v
Results.....	vi
Conclusions	vii
1 Introduction	1
1.1 Objectives.....	1
1.2 Description of the teams involved in microplastic research.....	1
1.2.1 Albania - Agricultural University of Tirana, Tirana	1
1.2.2 Croatia - Institute for Oceanography and Fisheries, Split	2
1.2.3 Italy - Regional Agency for Environmental Protection in the Emilia-Romagna region, Cesenatico.....	2
1.2.4 Slovenia	2
Institute for Water of the Republic of Slovenia, Ljubljana.....	2
National institute of Chemistry, Ljubljana	3
1.3 Literature review.....	3
2 Materials and methods	6
2.1 River outflow sampling and sample analysis	6
2.1.1 Albania	6
2.1.2 Croatia.....	7
2.1.3 Italy.....	7
2.1.4 Slovenia	9
3 Results.....	10
3.1 River outflow.....	10
3.1.1 Quantity of microplastic particles according to the number and weight.....	10
3.1.2 Comparison among all rivers	16
3.1.3 Size distribution	17
3.1.4 Identification of plastic type	17
4 Discussion.....	19
4.1 Interpretation of analytical results	19
4.2 Discussion of methodological problems	20
5 Conclusions	21
6 References	22

Derelict Fishing Gear management system in the Adriatic Region

Appendices.....	24
Appendix 1: Sampling data – river outflow.....	24
6.1.1 Albania	24
6.1.2 Croatia.....	24
6.1.3 Italy.....	25
6.1.4 Slovenia	26
Appendix 2: Size distribution – river outflow	27
6.1.5 Italy.....	27
6.1.6 Slovenia	28
Appendix 3: Po river discharge in years of 2014 and 2015.....	29



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Derelict Fishing Gear management system in the Adriatic Region

1 Introduction

This report presents the results of monitoring of microplastic and related activities in the work package 5 within the DeFishGear project - “Derelict Fishing Gear Management System in the Adriatic Region” (project code: 1°STR/ 00010), which was funded by IPA Adriatic Cross-border Cooperation Programme 2007 – 2013.

The report is focused exclusively on monitoring and assessment of microplastic pollution (plastic particles <5 mm) in the rivers that discharge into the Adriatic Sea. The report is a result of coordinated actions toward sharing knowledge on analysing microplastic pollution, which included application of the joint monitoring methodology in the Adriatic region aiming to determine microplastic types and quantities in coastal and marine departments.

1.1 Objectives

The main objectives of the output “Pilot assessment on microplastic in rivers” were:

- Selection of main rivers that outflow into the Adriatic Sea and where sampling activities could be done. Each country choose its largest river, except of Greece (eligible IPA Adriatic area – Corfu Island) and Bosnia and Herzegovina, since there are none rivers. Rivers included into the research were: Po in Italy, Dragonja in Slovenia, Neretva in Croatia, and Drin and canal Lumi Durrësit in Albania.
- Sampling on river outflows and sample analysis of water samples. Each country sampled at least 2 samples per river outflow.
- Estimation of microplastic concentration in the river outflow of selected rivers of Adriatic region.

1.2 Description of the teams involved in microplastic research

Output “Pilot assessment on microplastic in rivers” was carried out by:

- Regional Agency for Environmental Protection in the Emilia-Romagna region, Cesenatico, Italy;
- National Institute of Chemistry, Ljubljana, Slovenia;
- Institute for Water of the Republic of Slovenia, Ljubljana, Slovenia;
- Institute for Oceanography and Fisheries, Split, Croatia;
- Agricultural University of Tirana, Albania.

1.2.1 Albania - Agricultural University of Tirana, Tirana

The Agricultural University of Tirana (AUT) elaborates water management and marine management studies and technical background legal regulations which mainly support the Ministry of Agriculture and the Environment and develops approach to monitoring and management of marine litter pollution, including beach litter and microplastic and has been involved in Clean Coast, Marine Renegades and other NGO projects. The laboratory is involved in marine litter research since 2013 and has the leading role in microplastic analysis in Albanian sea water since 2013. AUT has also experience in socio-economic analysis (SEA).



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Derelict Fishing Gear management system in the Adriatic Region

1.2.2 Croatia - Institute for Oceanography and Fisheries, Split

The Institute of Oceanography and Fisheries (IOF) is the scientific public institute and it is National Reference Centre for the marine research. Institute employs 116 people of whom 71 are experts in different field of which 50 are professionals with PhD. Scientific activities conducted in the IOF are interdisciplinary including biological, chemical, physical, geological and fishery research. The research vessels Bios and Navicula, owned by IOF, are used for oceanographic and fisheries research activities, designed specifically for surveys and data collection for both coastal and open-sea waters.

The IOF have eight Departments dealing with variety aspects of coastal environment. Information available on the composition and distribution of marine litter, including microplastics in the Croatian marine environment is scarce because surveys to date have mainly focused on biological and hydrological parameters. During our long-term investigations, microplastics have been detected in the tissues of a variety of marine species, as well as in the water column and sediment. However, there is currently a lack of dedicated studies on the bio-ecological effects of various kind of marine litter on the sea life in Croatia. The IOF aims to participate in establishing a framework within which Policy makers and other stakeholders would take measures to achieve or maintain good environmental status in the sea.

The IOF team involved in DeFishGear project are employees of two laboratories; Laboratory of ichthyology and coastal fisheries and Laboratory of fisheries science and management of pelagic and demersal resources. The team includes scientists who are experts in the fields of biology and biotechnology, dealing with the biology and ecology of fishes and their developmental stages, trophic ecology, population dynamics, all aspects of fisheries including stock assessment and management of pelagic, demersal and coastal resources. At the beginning DeFishGear project, two people were hired to be fully involved in the activities assigned by the project and they were sent for training in order to specialize in collection and processing samples of microplastics.

1.2.3 Italy - Regional Agency for Environmental Protection in the Emilia-Romagna region, Cesenatico

Regional Agency for Environmental Protection in the Emili-Romagna region (ArpaER) carries out research about coastal marine systems with emphasis on multidisciplinary ecosystem complexity: the main activities include research, development and application of monitoring programme to comply with regional, national, EU laws. During the 2013 the ArpaER was involved in sea surface microplastics analysis for the Marine Strategy. In the 2015 they started again the activities about marine Strategy and we are involved in sea surface microplastics analysis. In Italy the microplastics analysis in the beach sediment are not take into consideration until now.

The team is composed from marine biologists and technicians that are involved in sampling activities and laboratory analysis with a couple years of experience in this skill.

1.2.4 Slovenia

Institute for Water of the Republic of Slovenia, Ljubljana

Institute for Water of the Republic of Slovenia (IWRS) implements common European water policy and international strategies and conventions, participates in expert working groups of the Common Implementation Strategy process of directives at the DG Environment of the European Commission and activities of the European Environment Agency. IWRS elaborates water management and marine management studies and technical background legal regulations which mainly support the Ministry of Agriculture and the Environment and develops approach to monitoring and management of marine



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Derelict Fishing Gear management system in the Adriatic Region

litter pollution, including beach litter and microplastic and has been involved in Clean Coast, Marine Renegades and other NGO projects.

The Institute is involved in marine litter research since 2010 and has the leading role in microplastic analysis in Slovenian sea water since 2011. The team is composed from biologists and technicians that are involved in sampling activities and laboratory analysis with a couple years of experience in this skill.

National institute of Chemistry, Ljubljana

The National Institute of Chemistry (NIC) is a public research institution active in all areas of chemistry. It was the first research institution in Slovenia to obtain ISO 9001 standards in 2003. It offers high-level research equipment such as NMR spectrometers, ultra-high resolution microscopes, chromatography systems etc., and is routinely involved in national and international research frameworks. The Department for Polymer Chemistry and Technology (D07) with its 17 members is specialized in synthesis and characterization of polymers and plastics. It provides a full array of chemical polymer/plastics characterization expertise: spectroscopy, chromatography, thermal methods, as well as an excellent staff including 9 Ph. D. experts.

The laboratory has a history of activity in the broad area of environmental aspects of plastics and polymers including plastic waste management, plastics recycling, biodegradability of plastics, biobased plastics and bionanocomposites. Through its project involvement D07 has expertise in plastics sustainability issues from its strong involvement in bioplastics as well as the legal, standardization/certification and strategic aspects of plastic waste management, plastic pollution in the environment and environmental solutions such as biodegradable plastics.

Through the waste management and biodegradability issues the team lead by dr. Andrej Kržan has had exposure to the issue of marine litter and microplastics. In cooperation with the University of Nova Gorica dr. Kržan lead the first studies of microplastics occurrence in the Adriatic sea (Slovenian part), which started in 2011 (two studies finished by 2015). The results of these exploratory studies were included in the national reporting as part of fulfilling the requirements for the Marine Strategy Framework Directive.

1.3 Literature review

The Adriatic Sea is north eastern part of the Mediterranean Sea, which separates the Apennine of the Balkan Peninsula. The Adriatic Sea is semi-enclosed sea connected with the Ionian Sea by the Strait of Otranto. It is geographically divided into the Northern Adriatic, Central (or Middle) Adriatic, and Southern Adriatic. The prevailing currents flow up from the Strait of Otranto, along the eastern coast and back to the strait along the western (Italian) coast. Because the Adriatic Sea collects a third of the fresh water flowing into the Mediterranean their salinity is lower than the average Mediterranean's salinity (Artegiani et al., 1997). The first contributor of freshwater to the Adriatic is the submarine groundwater discharges through submarine springs that comprise 29% of the total water flux into the Adriatic (Taniguchi et al., 2002). Among surface waters, river Po has the highest discharge into the Adriatic, followed by Neretva and Drin (Franić and Petrinc, 2006). Other major rivers discharging into the Adriatic include also Soča, Krka, Bojana, and Vjosë. Altogether with other small rivers discharge up to 5,700 m³/s. This rate of discharge amounts to 0.5% of the total Adriatic Sea volume.



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Rivers have important role in sediment deposition, where sediments are generally carried by the river on the northwest coast (Po, Reno, Adige, Brenta, Tagliamento, Piave and Soča). The volume of sediments carried from the eastern shore by the Rječina, Zrmanja, Krka, Cetina, Ombla, Dragonja, Mirna, Raša and Neretva rivers is negligible, because these sediments are mostly deposited at the river mouths. The western coast is alluvial or terraced, while the eastern coast is highly indented with pronounced karstification. The smooth Italian coast (with very few protrusions and no major islands) allows the Western Adriatic Current's smooth flow, which is composed of the surface's relatively freshwater mass and the bottom's cold and dense water mass.

In the project DeFishGear five river outflows were addressed for microplastic pollution, Po River in Italy, Dragonja River in Slovenia, Neretva River in Croatia, Drin and Lumi Durrësit rivers in Albania.

Po River is the largest river of the western part of the Adriatic basin and the largest river in Italy. It flows eastward across northern Italy and it is 682 km long and its average discharge is 1,540 m³/s. It discharges into the Adriatic near Venice with a wide delta with hundreds of small channels and five main ones, called Po di Maestra, Po della Pila, Po delle Tolle, Po di Gnocca and Po di Goro. The Po River flows through many important Italian cities, such as Torino, Piacenza and Ferrara, and since the Po valley is the main industrial area in Italy, the Po river is under high pressure of different kind of pollution, as heavy metals (Farkas et al., 2007), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), extractable organo halides (EOX) (Vigano et al., 2003) and plastic materials (van der Wall et al., 2015). On the Po river one study of macro and microplastic pollution was done in 2014, where was estimated that 677 milliard particles per year discharges into the Adriatic Sea by the river (van der Wall et al., 2015).

The Dragonja River is the largest river of Slovenian coast that outflow into the Adriatic Sea and the third longest river in Istria. It is 30 km long (Statistical Office of the Republic of Slovenia, 2002) and is a meandering river with a branched basin and a small quantity of water, which often dries up in the summer (Trobec, 2010). The mouth of the river is located in the Sečovlje Salina Landscape Park. Dragonja River has very good ecological status, since there is none of the industry.

Neretva is the largest river of the eastern part of the Adriatic basin, since it is 230 km length (208 km are in Bosnia and Herzegovina, while the final 22 kilometres in Croatia (Neretva river..., 2004). Neretva is the most important source of drinking water for Bosnia and Herzegovina as well as Croatia. The average discharge at profile Žitomislići in Bosnia and Herzegovina is 233 m³/s and at the mouth in Croatia 341 m³/s. Passing towns and villages in Bosnia and Herzegovina, the Neretva spills out into the Adriatic Sea, building a wetland delta that is listed under the Ramsar Convention as internationally important. In this lower valley in Croatia, the Neretva River splinters into multiple courses, creating a delta covering approximately 12,000 hectares. Neretva River is discharging into the Mali Ston bay, which is influenced also by several submarine springs situated inside the Bay. Its estuary is classified as the salt-wedge type, where due to small tidal currents the advection of the river water is much larger than the introduction of seawater through tidal mixing. Horizontal extension of the freshwater into the Channel mostly depends on the direction of local winds, and those can vary on a daily basis (Matić, 2005). Higher discharge of the submarine springs in winter causes a spreading of the less saline surface waters into the Neretva Channel and a compensatory inflow in the bottom layer. The strong NE "bora" wind can, however, reverse that circulation. During the summer, the circulation pattern depends entirely on the wind direction (Vučak et al. 1981).

The Drin is the longest river in Albania with a total length of 335 km of which 285 km flows through Albania with an average inflow of about 197 m³/s and two main branches (Black Drin, and then Drin



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downstream of the confluence with White Drin). In the North it collects water from the Adriatic portion of the Kosovo watershed and Montenegro (White Drin) and in the south from the border region with FYR Macedonia (Black Drin with drains Prespa Lake and Ohrid Lake) (Ose, 2009). The Drin River is the most constant river in Albania, fed by melting snow from the northern and eastern mountains and more frequent seasonal precipitation (Sogreah, 2008).

2 Materials and methods

2.1 River outflow sampling and sample analysis

In DeFishGear project five river outflows were sampled for microplastic analysis, the Po river outflow in Italy, Dragonja river outflow in Slovenia, Neretva river outflow in Croatia, and rivers Drin and Lumi Durrësit in Albania. The methodology for sampling and sample analysis was in all steps the same like for the sea surface sampling and is described in the document “Recommendation on regional approach to monitoring and assessment of microplastic in the marine environment” (Kovač Viršek et al., 2015) in the Chapter 2: Sea surface sampling and microplastic separation.

Individual descriptions of sampling sites are listed below with maps of sampling sites (Figure 1 - Figure 5). The technical data from individual river outflow samplings are in the Appendix 1, Table A1 - Table A5.

2.1.1 Albania

The samplings of river outflows were done on rivers Drin and canal Lumi Durrësit in January 2016. Samples were named R1 (1st sample), R2 (2nd sample) and R3 (3rd sample) (Figure 1 and Figure 2). The weather was sunny, and the wind was blowing in the direction of NW. Other technical data referred to this survey are described in Appendix 1, Table A1.

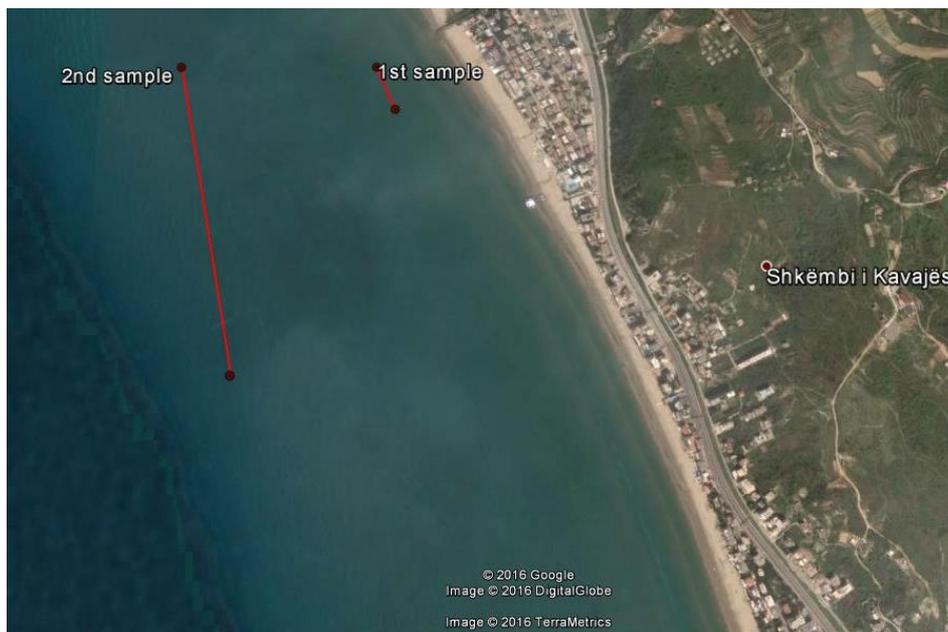


Figure 1: Map of sampling transects R1 and R2 from river outflow of Lumi Durrësit River on 26th and 27th of January 2016, Albania. Photo: Google Earth; Uroš Robič, IWRS.



Derelict Fishing Gear management system in the Adriatic Region

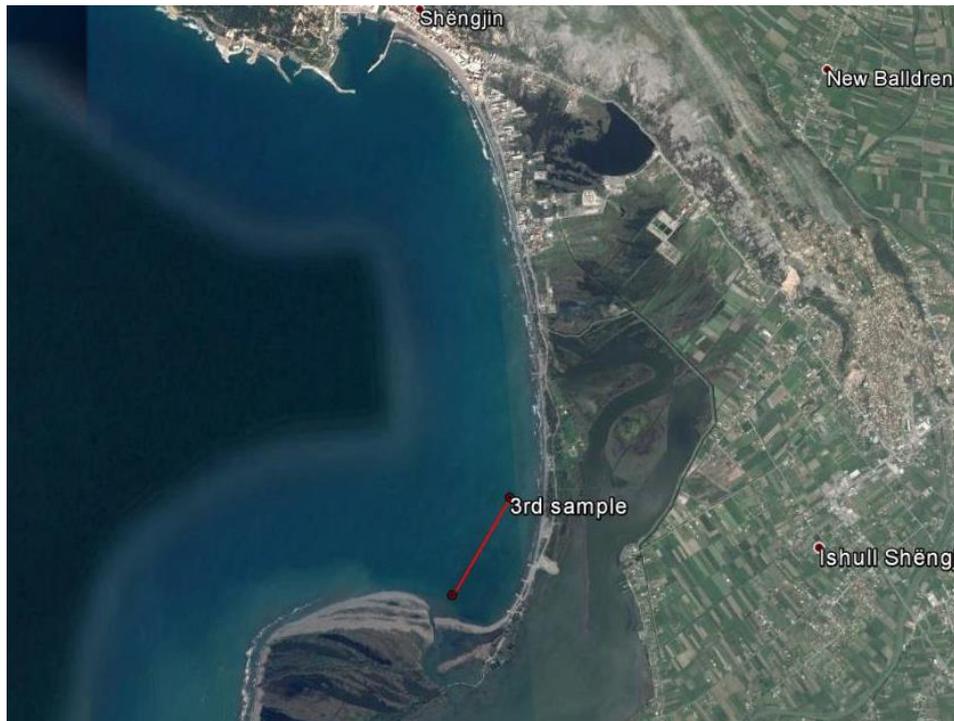


Figure 2: Map of sampling transects R1 and R2 from river outflow of Drin River on 28th of January 2016, Albania. Photo: Google Earth; Uroš Robič, IWRS.

2.1.2 Croatia

Five sea surface samples from mouth of Neretva River were taken in October 2014 (Figure 3). The other technical data are in Appendix 1, Table A2.



Figure 3: Map of river outflow sampling transects on 25th October 2014 in river outflow of Neretva River, Croatia. Photo: Google Earth; Uroš Robič, IWRS.

2.1.3 Italy

Three sampling stations (LN2_R, 302_R, 1002_R) were located close to the Po River mouth, in front of Lido delle Nazioni location (Figure 4). Transect was located close to the southern part of the delta,



Derelict Fishing Gear management system in the Adriatic Region

indeed strongly influenced by the Po di Goro. The coast is a sandy beach with Nord East coast orientation. Lido delle Nazioni is a small touristic town with a population of 2600 inhabitants. Generally, a strong increase of inhabitants are registered during summer months. The nearest port is Goro, a fishing harbour characterized by a 60 number of ships/year (distance approximately 10 km). The Po river outflow value registered on the 12th of December 2014 and on the 22nd of April 2015 were 2876 and 1394 m³/s, respectively. The Po river outflow annual mean was 2378 m³/s in 2014 and 1335 m³/s in 2015.

The samplings surveys were carried out on 12th December 2014 and 22nd April 2015 at Lido di Volano location (stations 2, 302, and 1002). The technical data referred to this surveys are described in Appendix 1, Table A3 and Table A4.



Figure 4: Map of river outflow sampling transects on 12th December 2014 and 22nd April 2015 at Lido di Volano location, stations 2, 302 and 1002, Italy. Photo: Google Earth; Uroš Robič, IWRS.



Derelict Fishing Gear management system in the Adriatic Region

2.1.4 Slovenia

The river outflow sampling was done on 25th of August 2014. Samples R1 and R2 were taken from transects made on border river Dragonja (Figure 5). The technical data from the samplings are in Appendix 1, Table A5.



Figure 5: Map of river outflow sampling transects on 25th of August 2014 on Dragonja River, Slovenia. Photo: Google Earth; Uroš Robič, IWRS.



3 Results

3.1 River outflow

3.1.1 Quantity of microplastic particles according to the number and weight

3.1.1.1 Albania

The amount of microplastic particles found in river outflow samples was the lowest in sample R2 (1,733,835 Items/km²) and the highest in sample R3 (1,952,959 Items/km²) (Figure 6). In average there were 1,857,346 particles per km² in the river outflow at the coast of Albania on the sampling days in January 2016.

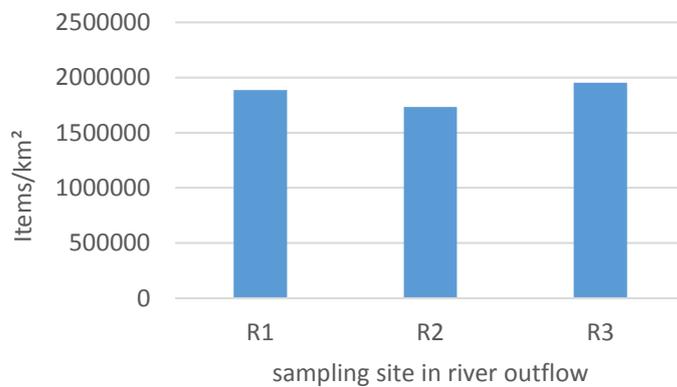


Figure 6: Microplastic concentrations (items/km²) in the river outflows for each transect from January 2016, Albania.

The weight of microplastics found in the river outflow samples followed different trend as observed for the number of microplastic. The weight of microplastic particles in sample R2 was lower than in sample R1 (Figure 7). The data for the weight of filaments is excluded from this comparison, since the weight of filaments wasn't established (due to the low weight of the filament particles and frequent contamination of filaments with non-plastic material). Also the data from the category "other" are excluded from this analysis.

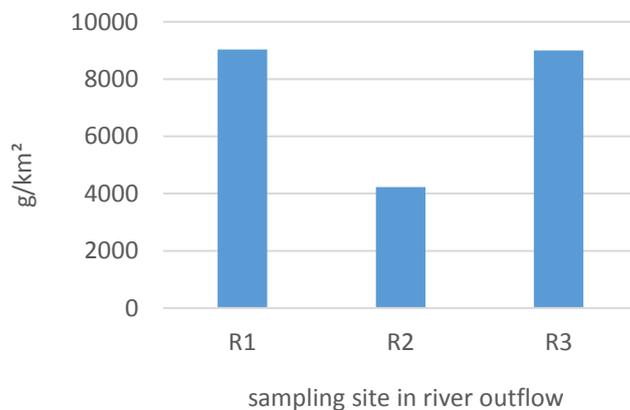


Figure 7: Weight of microplastic particles (g/km²) per sampling site from January 2016, Albania.

In all samples from river outflow, filaments were predominant type of microplastic categories, with an average around 60.8% (Figure 9). The second most common type of microplastic category were

Derelict Fishing Gear management system in the Adriatic Region

granules. Filaments were absent from sample R1 and fragments were absent from sample R2 (Figure 8).

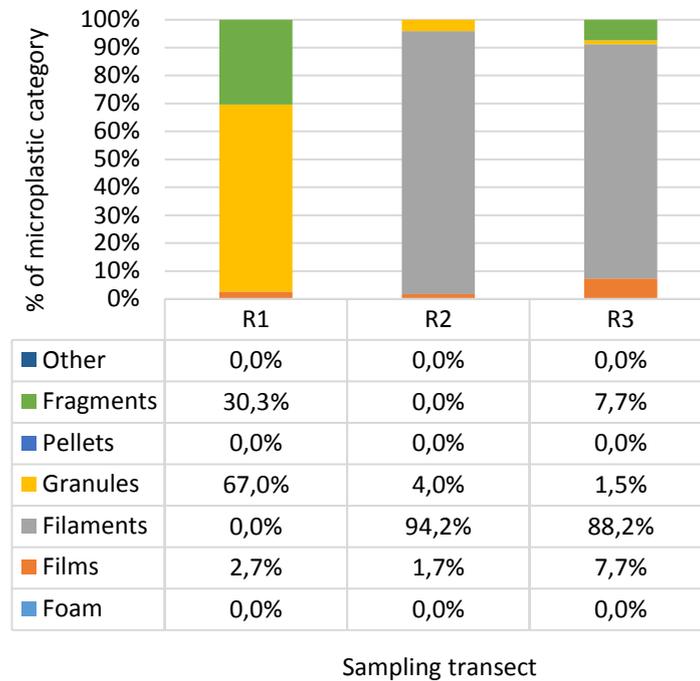


Figure 8: Composition of microplastic categories for each sample from January 2016, Albania.

3.1.1.2 Croatia (Sampling area: Neretva river outflow)

The average of microplastics concentrations on Neretva river outflow were $16.86 \times 10^4 \pm 11.36 \times 10^4$ particles per km^2 . The highest number of microplastic were found in locations that are nearest to the river bank. The highest amount of microplastics were found in sample N01 located in the inner part of river outflow. The concentration range was from 3.1×10^4 to 34.3×10^4 microplastic particles per km^2 (Figure 9). Representation of microplastics per category of river outflow were diverse from those of sea surface. The predominant category, except from N04 sample, were plastic fragments ranging from 49.48 - 70.50%. Second common category in all samples, except N04 sample, were filaments with maximum in N05 sample (40.00%). Films were found in all collected samples, except N05, and in N04 sample were predominant category with 44.05%. Foamed plastic were noted only in N01 sample with percentage of 18.40% (Figure 10).

Derelict Fishing Gear management system in the Adriatic Region

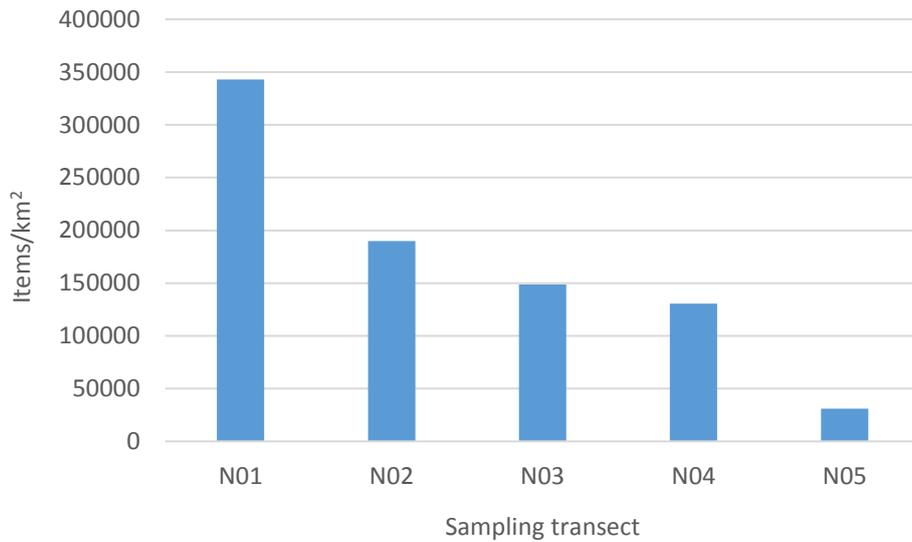


Figure 9: Number of microplastic particles per km² for 5 different transects from Neretva River outflow from autumn 2014, Croatia.

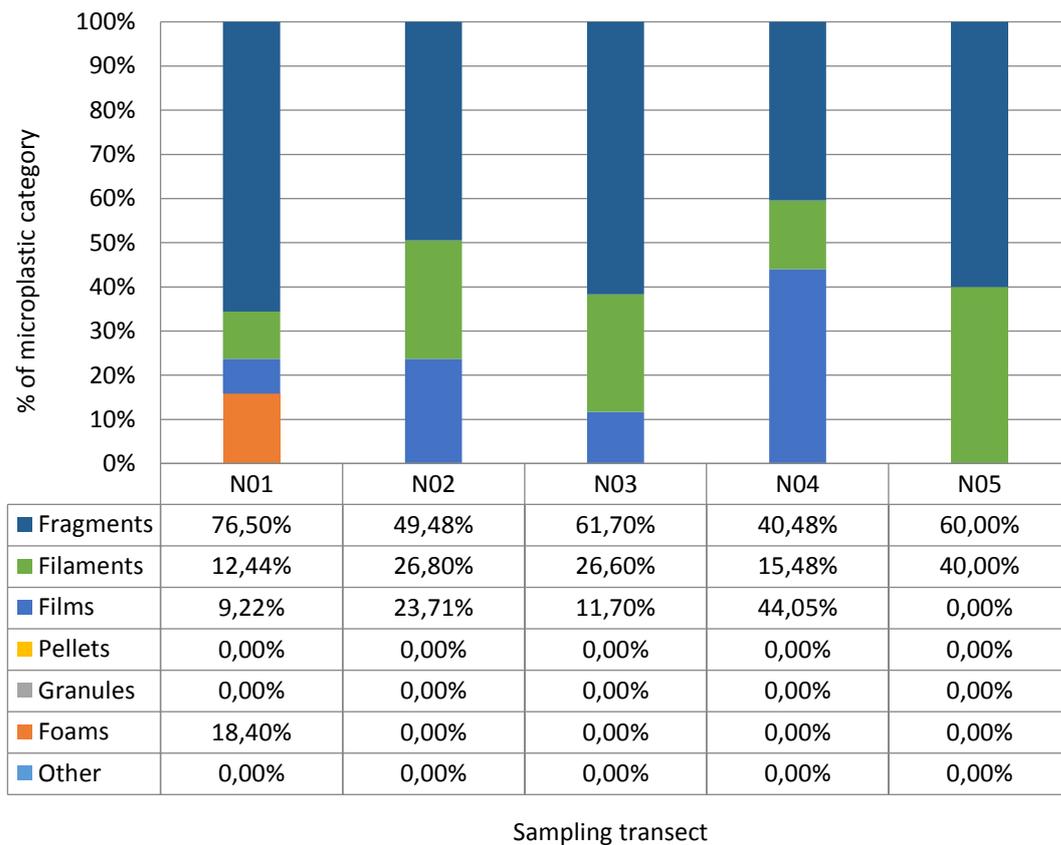


Figure 10: Composition of microplastic categories for each sampled transect from Neretva River outflow from autumn 2014, Croatia.

3.1.1.3 Italy (Sampling area: Po river outflow)

The quantity of microplastics sampled in the Po river outflow transect were higher during April 2015 compared to December 2014 in all the sampling stations (Figure 11). Moreover, the station situated

Derelict Fishing Gear management system in the Adriatic Region

at 10 km from the coast (1002_R) presented the highest amount of microplastics (744,000 Items/Km²), approximately 25 times higher compared to all the other stations. In fact, this area is strongly influenced by currents dynamics.

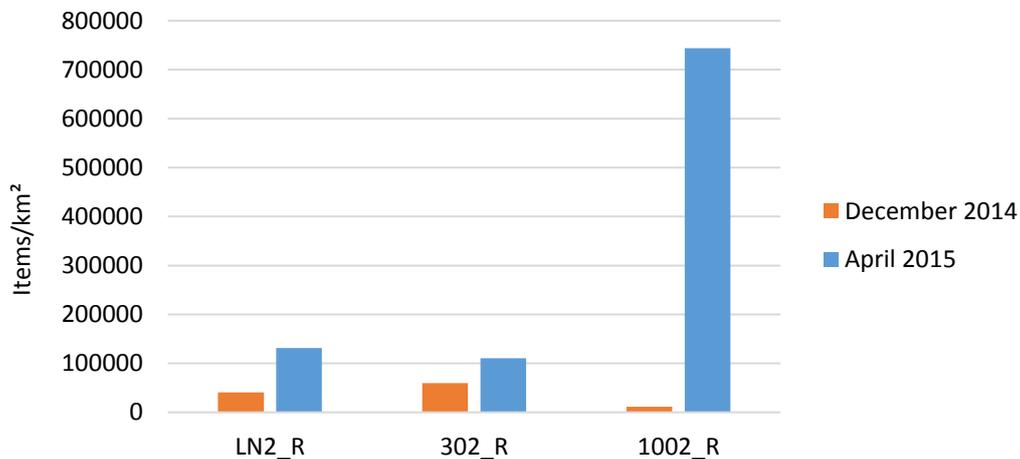


Figure 11: Number of microplastic particles per km² from 3 different transects from the Po River outflow from December 2014 and April 2015, Italy.

In December 2014, 8 g/km² of microplastics were detected in station LN2_R, and the quantity of microplastics decreased progressively with the distance from the river mouth (Figure 13). A 10 km distance from the coast (1002_R) the amount of plastic was significantly reduced (2.71 g/km²). On the contrary, in transect 14 the quantity of microplastic drastically increase at 10 km from the coast (up to 252.20 g/km²) compared to the stations closed to the river mouth during April 2015 (Figure 12).

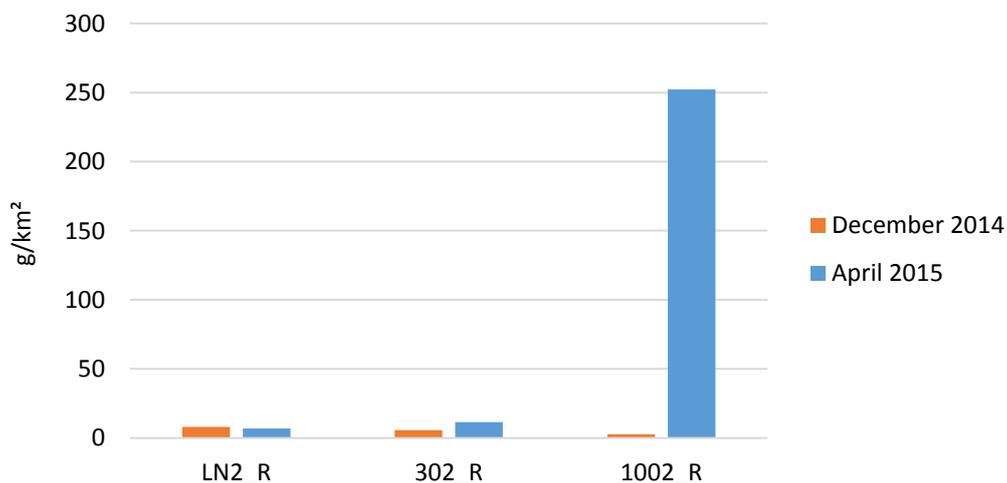


Figure 12: Weight of microplastic particles (g/km²) for transect 2 for the Po River outflow during December 2014 and April 2015, Italy.

During the two sampling campaigns the majority of the plastic particles analysed in the river outflow transect was constituted by fragments (71.08 - 84.53%) (Figure 13 and Figure 14). All the other plastic pieces belonged to films and filaments categories. Foam were detected only in the station 1002_R (11.11%) during April 2015.



Derelict Fishing Gear management system in the Adriatic Region

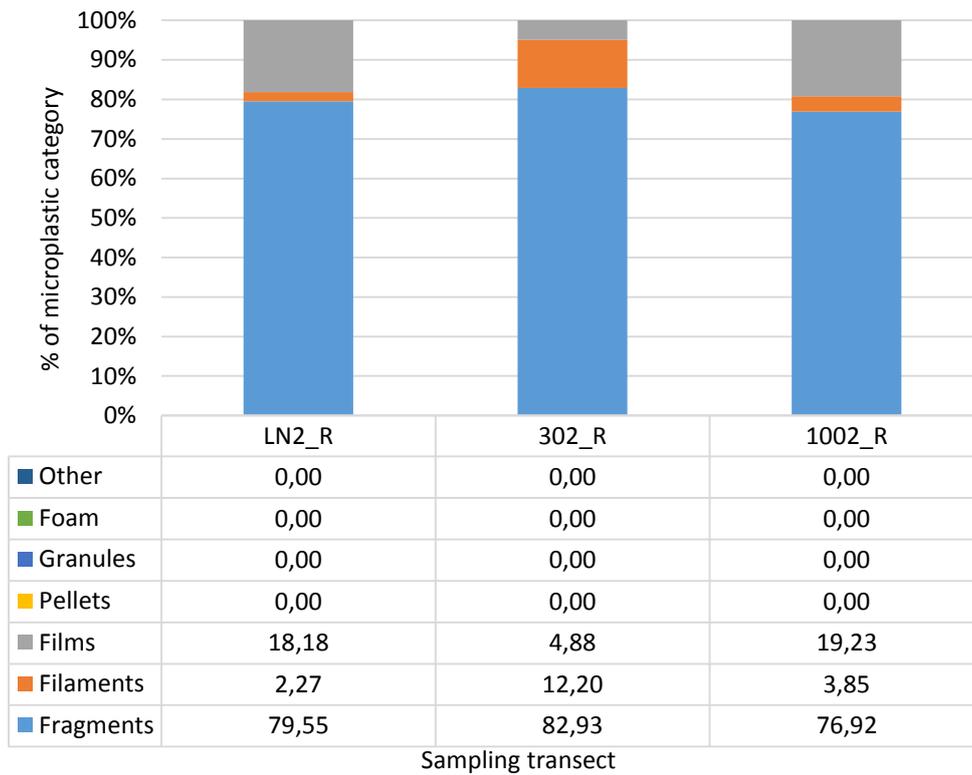


Figure 13: Composition of microplastic categories for transect 2 for the Po River outflow from December 2014, Italy.

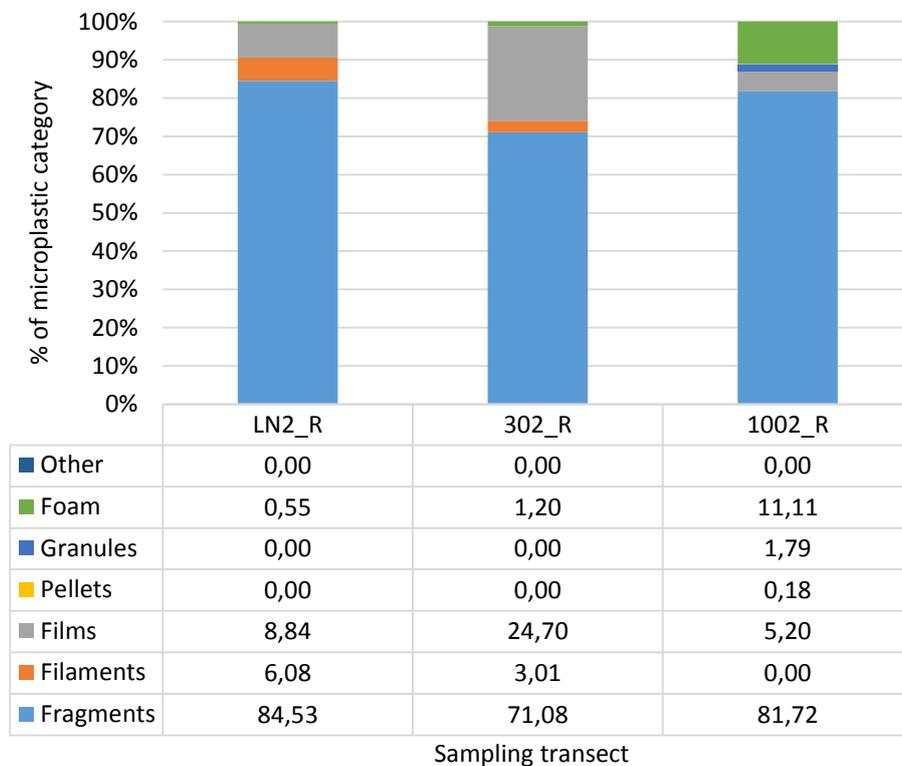


Figure 14: Composition of microplastic categories for transect 2 for the Po River outflow from April 2015, Italy.

Derelict Fishing Gear management system in the Adriatic Region

3.1.1.1 Slovenia (Sampling area: Dragonja river outflow)

The amount of microplastic particles found in river outflow samples was the lowest in sample R1 (196,914 Items/km²), and the highest in sample R2 (226,257 Items/km²) (Figure 15). In average there were 211,585 ± 20,748 particles per km² in the river outflow at the coast of Slovenia on the sampling days in August 2014.

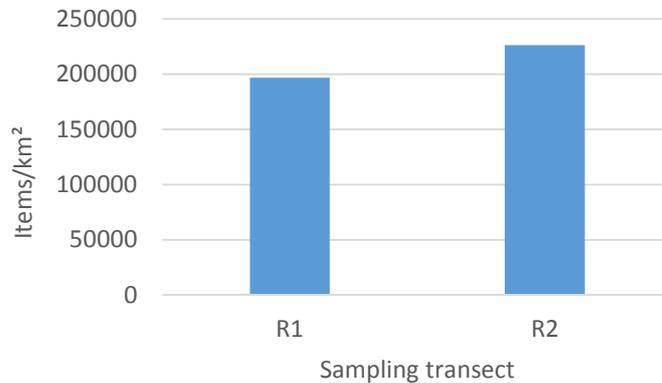


Figure 15: Number of microplastic particles per km² per transect of Dragonja River outflow from August 2014, Slovenia.

The weight of the microplastic particles found in the river outflow samples followed different trend as observed for the number of microplastic. The weight of microplastic particles in sample R2 was lower than in sample R1 (Figure 16). The data for the weight of filaments is excluded from this comparison, since the weight of filaments wasn't established (due to the low weight of the filament particles and frequent contamination of filaments with non-plastic material). Also the data from the category "other" are excluded from this analysis.

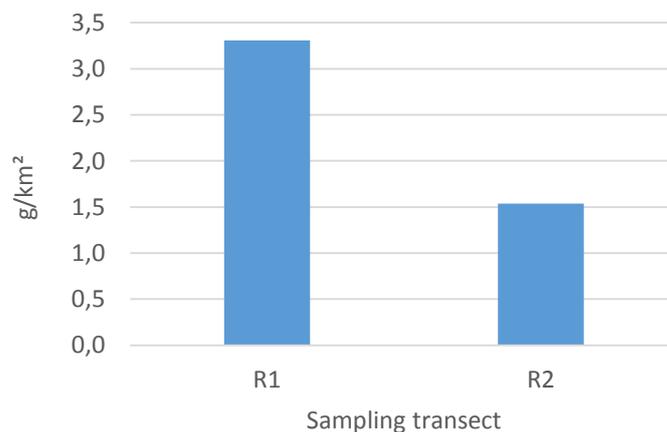


Figure 16: Weight of microplastic particles (g/km²) per transect of Dragonja River outflow from August 2014, Slovenia.

In both samples from river outflow, filaments were predominant type among microplastic categories, with an average around 80% (Figure 18). The second most common type of microplastic category were fragments. Granules were absent from sample R1 and pellets were absent from sample R2 (Figure 17).

Derelict Fishing Gear management system in the Adriatic Region

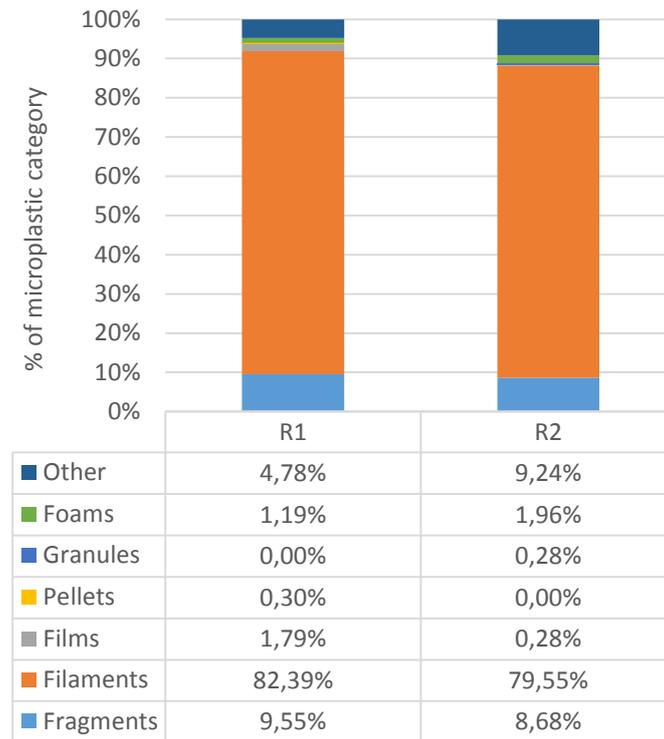


Figure 17: Composition of microplastic categories for each transect of Dragonja River outflow from August 2014, Slovenia.

3.1.2 Comparison among all rivers

The comparison among Po, Neretva and Dragonja river outflows show the similar average concentrations of microplastic, but the microplastic concentration on the Drin and Lumi Durrësit is almost ten times higher than in the other three rivers (Figure 18). Therefore Albanian rivers had the highest average concentration (1,857,346 Items/km²) and Neretva River had the lowest concentration (168,600 Items/km²). The concentration range was the largest for the Po River (11,000 – 744,000 Items/km²) and the smallest for the Dragonja River (197,000 – 226,000 Items/km²).

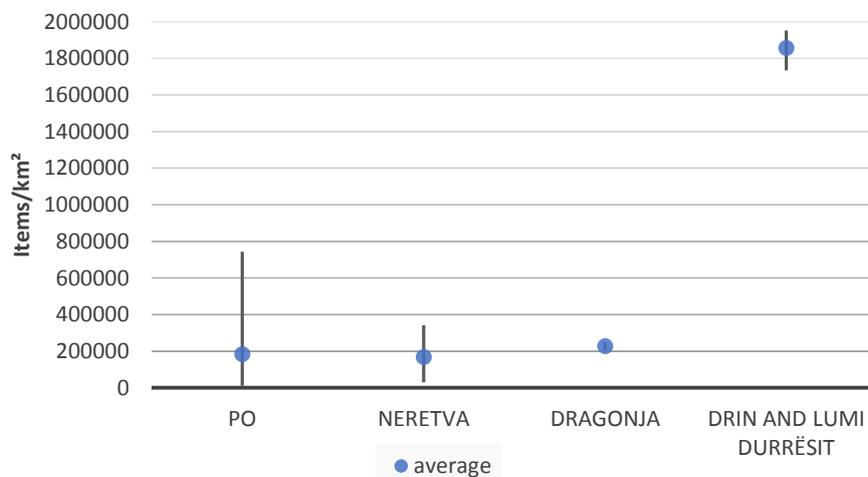


Figure 18: Average number of microplastic particles per km² from all samplings of river outflow on rivers Po in Italy, Dragonja in Slovenia, Neretva in Croatia, and Drin and Lumi Durrësit in Albania in years from 2014 to 2016.

Derelict Fishing Gear management system in the Adriatic Region

3.1.3 Size distribution

Particles sizes were measured for Italian and Slovenian samples, where particles from categories fragments, films, foams, pellets and granules were found. The average size of microplastic particles of Italian samples was 1.96 ± 1.12 and for Slovenian was 2.07 ± 1.69 . In Italy and Slovenia particles were very variable in length, since their measurements were in range from 0.32 to 5.09 in Italy and from 0.23 up to 10.23 mm in Slovenia. All results are presented in Appendix 2, Table A6 - Table A8.

3.1.4 Identification of plastic type

3.1.4.1 Croatia

Some of particles from Neretva outflow were processed and from those, 90% of microplastic particles were from polyethylene (PE), and 10% of particles were from polypropylene (PP) (Figure 19).

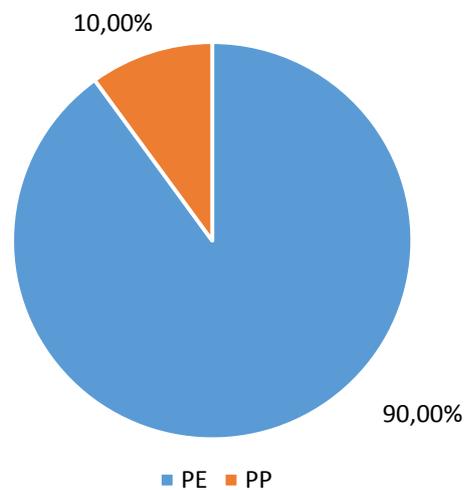


Figure 19: Polymer composition of films separated from sea surface samples taken on Neretva River outflow (PE – Polyethylene; PP – polypropylene), Croatia.

3.1.4.2 Italy

From processed particles from Po River outflow 60% were from nylon (NY), 20% were from polyethylene, 20% from polyamide (Figure 20).

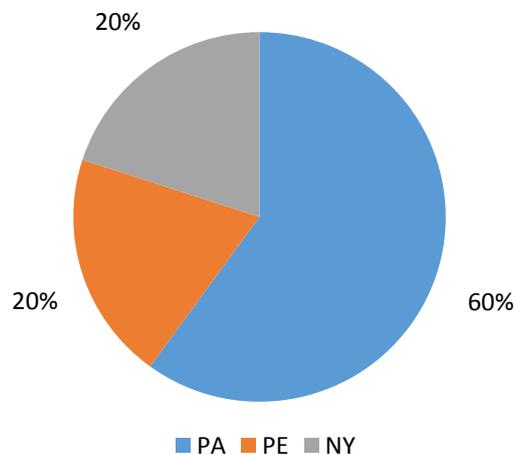


Figure 20: Chemical composition of microplastic particles for the sample 302 from 12th of December 2014, Italy.

Derelict Fishing Gear management system in the Adriatic Region

3.1.4.3 Slovenia

Chemical analysis of microplastic particles revealed that polyethylene (PE) and polypropylene (PP) were the most common type of plastic in both river outflow samples (Figure 21 and Figure 22). There was more than 60% of PE and more than 20% PP in the measured samples. Other types of plastic compounds found were polyamide (PA), polystyrene (PS), polyethylene terephthalate (PET) and PA+PE composite.

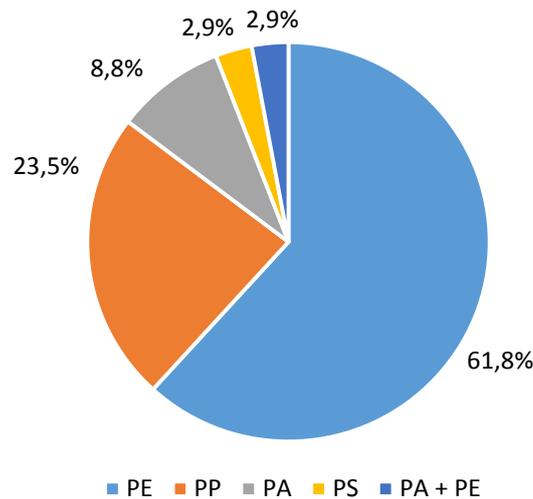


Figure 21: Chemical composition of microplastic particles for the Dragonja outflow river sample R1, Slovenia.

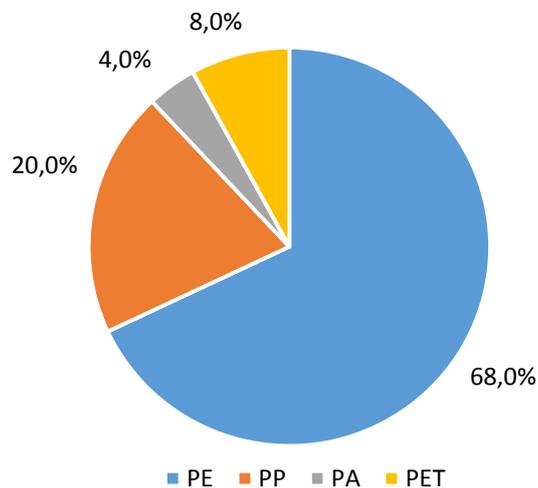


Figure 22: Chemical composition of microplastic particles for the Dragonja outflow river sample R2, Slovenia.



4 Discussion

4.1 Interpretation of analytical results

Results of the analysis offer a view on microplastics (300 μm - 5 mm) in five riverine outflows that discharge into the Adriatic Sea. The first observation from these results is that all riverine outflows are polluted by microplastic particles. Concentrations on outflows on rivers Po, Dragonja and Neretva are very similar, without statistical significant difference among them. Namely, the mean concentration on the Neretva outflow was measured as 168,000 Items/ $\text{km}^2 \pm 113,600$ Items/ km^2 , on the Po outflow as 182,000 $\pm 278,000$ Items/ km^2 , and on the Dragonja outflow as 226,000 $\pm 21,000$ Items/ km^2 . On Neretva and Po river outflows sampled in December 2014, the samples taken in inner part of outflow had higher concentrations of microplastic (Figures 10 and 12, respectively), while this could not be observed on the outflow of Dragonja river (Figure 16).

The results of Dragonja outflow are similar for the whole Piran Bay. The reasons for this could be that Dragonja River is short and has smaller quantity of water, especially in the summer, when sampling was performed. Also the outflow area of Dragonja River is small and has therefore small impact on the rest of the sea water in the bay. While Po and Neretva rivers are the largest rivers that discharges into the Adriatic Sea and thus have bigger impact on microplastic pollution. The quantity of microplastic particles discharged by Po River into the Adriatic Sea was already calculated in the project "Identification and Assessment of Riverine Input of (Marine) Litter" founded by European Commission DG Environment under Framework Contract No ENV.D.2/FRA/2012/0025. The measured concentration of microplastic was 2 million particles per km^2 . While the discharge from the Po River was 2 times higher during winter 2014 compared to the spring season 2015 (Appendix 3, Figures A1 and A2), the microplastic concentrations were higher in spring season, we can conclude that the concentrations on the riverine outflow are diluted, since this area is strongly influenced by dynamics of local sea currents.

Among microplastic categories, the number of particles in category of fragments was the highest (>50% of all founded particles). Fragments were the most prevalent particles in the Po and Neretva river outflows, while in the Dragonja River, filaments were the most numerous group of particles. If film particles, which in fact also represent the fragments, are added to the number of fragments this class is even higher with total of >60% of all particles collected. Fragments and film particles are, in the most cases, the result of fragmentation of larger plastic items (secondary source of origin). Whereas the Po and Neretva rivers flows through the large cities and industrial zones (especially Po River) the large concentrations of fragments are expected. In comparison with Neretva and Po River, Dragonja River is very short and flows through the small villages where there is no industry. The only source of pollution are discharges from households, where filaments from washing machines are the major part of microplastic pollution.

Very interesting results is, that among microplastic categories, there were no pellets found, also on the Po outflow, while on the river Po (near Ferrara) there was high concentrations of pellets found ($\sim 200,000$ Items/ km^2) in already mentioned research in this area in 2014 (Van der Wall et al., 2015). The reason could lie in lateral mixing of riverine flow of Po River and local sea currents that can cause the transfer of pellets under the sea surface. The reason could also be higher mass of pellets as particles in comparison to fragments and filaments, that can cause sinking of pellets on the bottom of the sea.

Results of chemical analysis of particles were similar for all measured samples, with polyethylene as the most abundant material in Slovenia, followed by polypropylene, and with polyamide (nylon) in Italy. This result was expected. Namely, polyethylene is the most commonly used plastic polymer in



Derelict Fishing Gear management system in the Adriatic Region

the world because it is strong, light, tough, resistant to acids, alkaline and other organic solvents and resistant to higher temperatures. It is an essential material for power transmission, food packaging, consumer goods, electronics, household goods, industrial storage, and transportation industries. The second reason for this result is the fact that polyethylene and polypropylene have very low densities and thus float on water and are therefore highly mobile. We believe that the combination of large quantities and the mobility due to floating leads to the observed situation in the Adriatic Region. Also polyamide, as it is nylon, is common material used. It is known for its good chemical resistance, excellent technical properties, and thermal stability. Nylon is widely used in fishing sector as material for fishing nets, therefore the high occurrence of microplastics from that material is expected in areas with extensive fishing activities.

4.2 Discussion of methodological problems

Samplings on the river outflows were performed by the use of manta net and the protocol for the sea surface sampling (Recommendation on regional approach..., 2015). One of the important factors that influenced the quality of sampling and therefore the results, is microlocation of sampling transects and direction of the boat's movement. In our case, movement of the boat was in the same direction like riverine flow. Our suggestion is that more microplastic particles will be caught if ship will move in opposite direction to the riverine flow and that microlocation is in front of the main branch of the river, if river has larger delta.

Seasonal conditions play a critical role in the transport of microplastic by the river. Precipitation in the entire watershed transports the dispersed litter on land through different pathways to the main water channel, where it is transported to the sea. At the beginning of the wetter season, the watershed gets cleaned and stored litter gets flushed away. This is a known phenomenon and it is called "hysteresis". If sampling is performed in different seasons, like in Italy, it is very important that weather conditions are as similar as possible, since only in this case the results can be compared. The most important is wind who influence on surface currents.

In the process of separation of microplastic from the collected samples, we determined important factors that can influence on the final results of microplastic concentration in the sample. These factors are:

- The contamination of the sample with air born filaments in the time of separation of microplastic from the sample. For this reason in all steps the use of blank samples is recommended and if possible the use of clean room or a laminar airflow cabinet.
- When samples are full of organic matter, the degradation of organic matter by 30% hydrogen peroxide is recommended, but without heating above 30°C.

5 Conclusions

On the basis of the first results of microplastic pollution on riverine outflows in Adriatic region, the following conclusions were made:

- The comparison among Po, Neretva and Dragonja river outflows showed similar average concentrations of microplastics with a range from 168,600 to 228,046 Items/km², but the concentration of particles in Albanian rivers was almost 10 times higher than in the other three rivers (1,857,346 Items/km²).
- The most prevalent category of particles were fragments in the Po and Neretva river outflow, and filaments in Dragonja, Drin and Lumi Durrësit river outflow. Interestingly, no pellets were found.
- The most abundant plastic material among microplastic particles sampled from river outflows of Adriatic Sea was polyamide (nylon) in Italy and polyethylene in Slovenia.
- In order to achieve better results, the use of blank samples at all times during the sample preparation and sample analysis is advised to consider the airborne contamination of fibers. Also the use of clean room or laminar air flow cabinet are welcomed. The use of 30% hydrogen peroxide is recommended, but without heating above 30°C.
- This research, conducted in the frame of DeFishGear project, present the first valuable data on the overall state of the contamination with microplastic in years from 2014 to 2016 on rivers Po, Dragonja, Neretva, Drin and Lumi Durrësit that discharges into the Adriatic Sea.



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Derelict Fishing Gear management system in the Adriatic Region

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Appendices

Appendix 1: Sampling data – river outflow

6.1.1 Albania

Table A1: Technical data for river outflow sampling on 26th, 27th and 28th of January 2016 in river outflow of Drin and Lumi Durrësit rivers, Albania.

Manta net samples		1st sample	2nd sample	3th sample
Sample name		R1	R2	R3
Start point	Time	12:10	11:20	12:12
	Lat (Y)	41.28429	41.2846	41.77566
	Lon (X)	19.50663	19.49961	19.59903
Stop point	Time	12:42	11:56	12:43
	Lat (Y)	41.28302	41.27603	41.76911
	Lon (X)	19.50725	19.5015	19.5932
Average speed (kn)		2.2	2.4	2.6
Transect length (nmi)		1.438	1.313	1.563

6.1.2 Croatia

Table A2: Technical data for river outflow sampling on 25th October 2014 in river outflow of Neretva River, Croatia.

Sampling date	Weather conditions (rainy/sunny/cloudy)				
25.10.2014	Sunny				
Sample ID	N01	N02	N03	N04	N05
Start point TIME	14:20	14:45	15:10	15:35	16:00
Start point GPS Lat (N)	43° 2'10.51"	43° 1'37.31"	43° 1'30.31"	43° 1'14.49"	43° 1'9.77"
Start point GPS Lon (E)	17°26'31.15"	17°26'28.63"	17°25'56.07"	17°26'35.59"	17°26'39.54"
Stop point TIME	14:40	15:05	15:30	15:55	16:20
Stop point GPS Lat (N)	43° 1'39.25"	43° 1'29.91"	43° 1'12.10"	43° 1'25.48"	43° 0'56.42"
Stop point GPS Lon (E)	17°26'30.34"	17°26'4.48"	17°26'35.63"	17°26'12.54"	17°26'12.46"
Average speed (kn)	2	2	2	2	2
Transect length (nm)	0.57	0.46	0.57	0.58	0.58
Sea state (0 - 9 B)	0	0	0	0	0
Wind velocity (1 - 12 B)	1	1	1	1	1
Wind direction (°)	NE	NE	NE	NE	NE

Derelict Fishing Gear management system in the Adriatic Region

6.1.3 Italy

Table A3: Technical data for river outflow sampling on Lido di volano on 12th December 2014 in river outflow of Po River, Italy.

Sample ID	2	302	1002
Start point TIME	10:00	10:27	11:02
Start point GPS Lat (N)	44°45.143	44°45.459	44°45.151
Start point GPS Lon (E)	12°15.480	12°17.374	12°22.681
Stop point TIME	10:20	10:47	11:22
Stop point GPS Lat (N)	44°46.363	44°45.480	44°44.965
Stop point GPS Lon (E)	12°16.850	12°19.255	12°25.537
Transect length (nm)	0.98	0.62	2.06
Sea state (0 - 9 D)	1	1	2
Wind velocity (1 - 12 B)	1	1	2
Wind direction (°)	NW	NW	NW

Table A4: Technical data for river outflow sampling on Lido di volano on 22nd April 2015 in river outflow of Po River, Italy.

Sample ID	2	302	1002
Start point TIME	11:25	10:40	09:43
Start point GPS Lat (N)	44°45.350	44°45.784	44°45.747
Start point GPS Lon (E)	12°15.593	12°17.143	12°22.489
Stop point TIME	11:45	11:10	10:03
Stop point GPS Lat (N)	44°44.137	44°45.386	44°44.838
Stop point GPS Lon (E)	12°15.289	12°18.980	12°25.948
Average speed (kn)	3.8 knots	4.1 knots	4.2 knots
Transect length (nm)	2.3	2.5	2.5
Sea state (0 - 9 D)	0	2	2
Wind velocity (1 - 12 B)	0	2	2
Wind direction (°)	/	NW	NW

Derelict Fishing Gear management system in the Adriatic Region

6.1.4 Slovenia

Table A5: Technical data for sampling of river outflow on 25th of august 2014 on Dragonja River, Slovenia.

Sample ID		R1	R2
Sampling date		25/8/2014	25/8/2014
Start point	Time	8:30	9:35
	Lat (Y)	45°30'29.0``	45°28'87.8``
	Lon (X)	13°35'15.8``	13°34'85.1``
Stop point	Time	9:01	10:05
	Lat (Y)	45°29'08.8``	45°29'87.8``
	Lon (X)	13°34'60.3``	13°33'70.0``
Average speed (kn)		2,7	2,5
Transect length (nmi)		1,415	1,289

Appendix 2: Size distribution – river outflow

6.1.5 Italy

Table A6: Results of image analyses: average, standard deviation, maximum and minimum length of the different microplastic categories collected in the river outflow sampling transects on Po River for the year 2014, Italy.

Data	Sample name	Category	Items of particles 5-0.3mm	Average length \pm SD (mm)	Max length (mm)	Min length (mm)
2014-12-12	LN2_R	Fragments	35	1.07 \pm 0.74	4.39	0.32
2014-12-12	LN2_R	Filaments	1	-	-	-
2014-12-12	LN2_R	Films	8	2.38 \pm 1.28	5.09	1.11
2014-12-12	302_R	Fragments	34	0.91 \pm 0.59	2.81	0.3
2014-12-12	302_R	Filaments	5	-	-	-
2014-12-12	302_R	Films	2	0.88 \pm 0.17	1	0.76
2014-12-12	1002_R	Fragments	20	1.36 \pm 0.96	4.52	0.53
2014-12-12	1002_R	Filaments	1	-	-	-
2014-12-12	1002_R	Films	5	1.38 \pm 1.42	3.82	0.34

Table A7: Results of image analyses: average, standard deviation, maximum and minimum length of the different microplastic categories collected in the sampling transects on Po River for the year 2015, Italy.

Data	Sample name	Category	Items of particles 5-0.3mm	Average length \pm SD (mm)	Max length (mm)	Min length (mm)
2015-04-22	LN2_R	Fragments	153	1.02 \pm 0.72	0.2	4.73
2015-04-22	LN2_R	Filaments	11	2.43 \pm 2.02	0.78	7.91
2015-04-22	LN2_R	Films	16	2.60 \pm 2.03	0.59	7.94
2015-04-22	LN2_R	Foam	1	-	-	-
2015-04-22	302_R	Fragments	118	1.12 \pm 0.68	0.29	4.22
2015-04-22	302_R	Filaments	5	2.80 \pm 1.38	1.41	4.82
2015-04-22	302_R	Films	41	2.53 \pm 1.96	0.71	7.88
2015-04-22	302_R	Foam	2	4.28 \pm 1.96	2.9	5.66
2015-04-22	1002_R	Fragments	912	1.47 \pm 1.23	0.22	9.72
2015-04-22	1002_R	Films	58	2.57 \pm 1.37	0.55	6.48
2015-04-22	1002_R	Pellets	2	4.24 \pm 0.31	4.02	4.46
2015-04-22	1002_R	Granules	20	0.67 \pm 0.27	0.22	1.48
2015-04-22	1002_R	Foam	124	1.65 \pm 0.99	0.42	5.38

Derelict Fishing Gear management system in the Adriatic Region

6.1.6 Slovenia

Table A8: Results of image analyses: number of particles in each category and size fraction, average length with standard deviation, maximum and minimum length of particles [mm] in samples from august 2014, Slovenia.

Sample name	Category	Items of particles < 1mm	Items of particles 1 - 5mm	Items of particles > 5mm	Average length (mm)	Max length (mm)	Min length (mm)
R1	fragments	12	19	1	1,64 ± 1,34	5,75	0,38
	films	1	3	2	4,61 ± 4,00	10,23	0,89
	foam	1	3	-	2,49 ± 1,35	3,85	0,93
	pellet	-	1	-	0,43		
	other	4	11	1	2,11 ± 1,44	6,26	0,50
R2	fragments	21	10	-	1,21 ± 1,08	4,01	0,23
	films	1	-	-	0,98		
	foam	2	5	-	1,28 ± 0,54	2,11	0,66
	granules	1	-	-	0,51		
	other	-	1	2	5,49 ± 2,10	7,77	3,64



DeFishGear

Derelict Fishing Gear management system in the Adriatic Region

Appendix 3: Po river discharge in years of 2014 and 2015

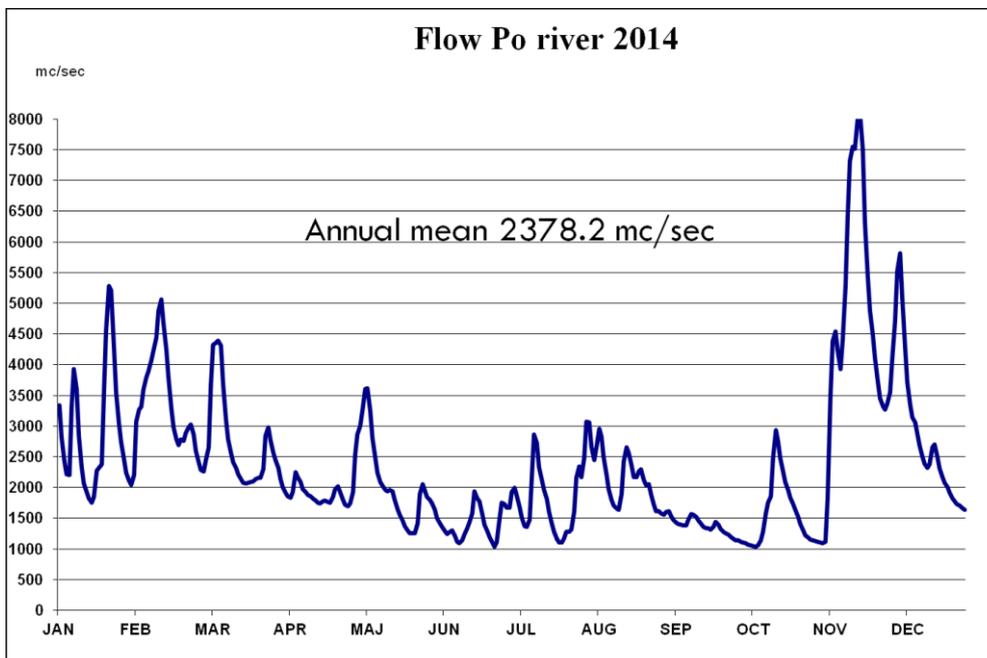


Figure A1: Po River discharge in 2014, Italy.

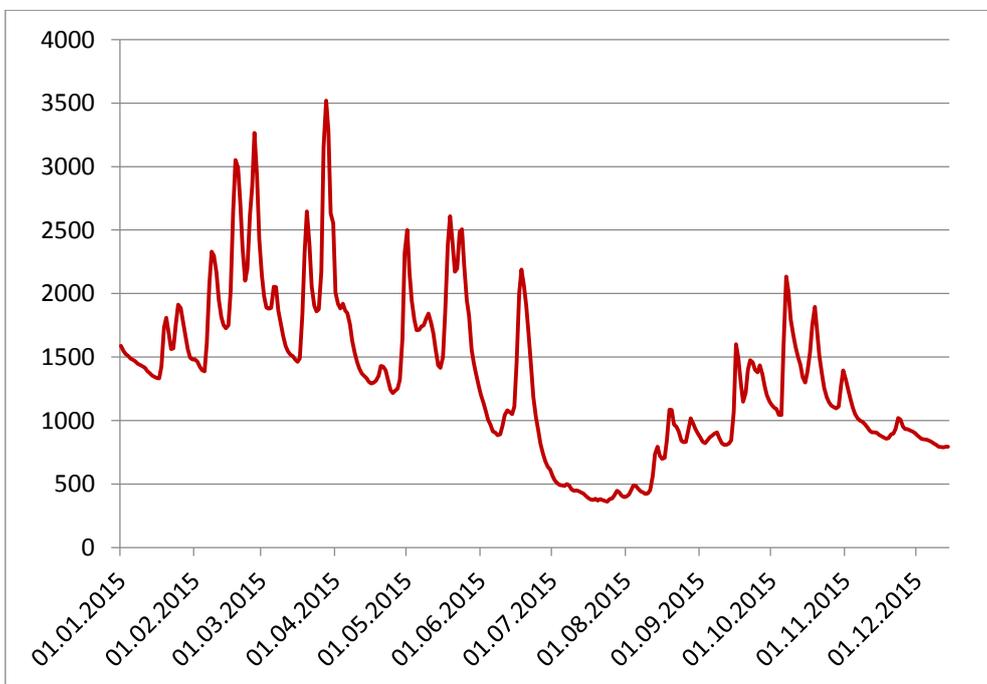


Figure A2: Po River discharge in 2015, Italy.