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Plastic pollution in aquatic systems

Mémoire présenté par

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Summary

Since the mass production of plastics began in the 1940s, plastic has achieved a pivotal status, with extensive commercial, industrial, medicinal and municipal applications. However, as the super resistance and persistence nature of the plastic, especially the plastic micro beads, it poses a massive contamination problem for the environment, especially in the aquatic system where it is difficult to monitor.

Here, this research has been conducted mainly with the following objectives: (1) Characterization of positive and negative plastic latex in Milli-Q water, natural aquatic systems and culture media. (2) microplastic ecotoxicity test with *Daphnia magna*. (3) Sampling, isolation and characterization of plastics from Geneva Lake.

This study is hoped to be a first step towards better understanding and addressing mainly the micro-plastic pollution problem in different aquatic systems. By integrating the literature study with the laboratory experiments of the plastic pollutants, I expect this work will provide valuable insight into determining the influence of the plastic to the environment, and that it will also offer a starting point for stakeholders, and society more broadly, to develop community-centered initiatives to reduce or even eliminate plastic pollution in our environment.

Key words: plastic pollutants, micro beads, microplastics, Plastic debris, ecotoxicity

1. Introduction

In contemporary society, along with the development of technology, more and more synthetic plastics have posed a massive contamination problem for the environment, especially in the aquatic system where it is difficult to monitor. Once plastics reach the marine and other aquatic environment, they fragment into smaller and smaller pieces following a series mechanical, chemical and biological processes. For example, under the effects of thermal UV degradation and hydrolysis, photodegraded and oxidized plastic debris gradually becomes brittle (Cooper and Corcoran, 2010)¹.

Due to chemical weathering and mechanical processes, the plastic fragment will be transformed into micro-plastic with different sizes. Microplastics are now everywhere in the marine environment at the surface and at depth in oceans and coastlines from the equator to the poles. Despite the growing number of publications on this topic, large gaps still remain in our understanding of the different plastic source, transport and fate of microplastics in the marine environment (Desforges et al., 2014). As result, plastic debris has become increasingly recognized as a global wide problem, allowing particles to persist for estimated years to millennia.

Moreover, plastic mulching could be another terrestrial source of microplastics but these pathways have not yet been sufficiently documented. One study found synthetic fibers in several soils in the U.S. to which organic waste material had been applied (Zubris and Richards, 2005). some other studies have just reported the presence of plastic in soil, but have not quantified the amount, nor described the size of the plastic particles (Rillig, 2012). Once in the soil, these plastic particles may persist, accumulate, and eventually reach levels that can affect the functioning and biodiversity of the soil and the terrestrial ecosystems (Rillig, 2012).

For the macroplastics, once they reach the marine environment, they fragment into smaller and smaller pieces following a number of mechanical, chemical and biological processes as we said before. Poly-particles (PE, PP or PS) with different sizes were first considered. These particles are used as model plastic to get an insight into their surface properties and stability in various environments representative of aquatic systems. Firstly, we measured the characterization of plastic latexes in natural freshwater and ultra pure water with different pH and I (It is salinity which is the quantity of dissolved salt content of the water. Salts are compounds like sodium chloride, magnesium sulfate, potassium nitrate, and sodium bicarbonate which dissolve into ions.). Precisely, we analyzed the stability of plastics in different solution with the pH value between 3 to 10. In this case, we also measured the surface charge variation via the measurement of the zeta potential. Because of the positive charge and negative charge of particles, it made the particles adhere on the surface of the plastic particles and change the density of the particles.

Based on the first step, we investigated the behavior of the plastic microbeads in presence of NOM (natural organic matter) at different conditions. The influence of NOM adsorption at the surface of the microbeads will be investigated. For example, when these different particles (plastic microbeads and the NOM) introduced into the different water conditions with different pH rate, what kind of reaction or behavior maybe happen between them? The density of the plastic microbeads, as well as the plastic surface properties, will be changed evidently during this period.

With previous research, the coming step we will analyze the impact of the plastic microbeads to the aquatic organisms, meanwhile, the ecotoxicity of the microplastics to the natural organisms. The aquatic organisms will be exposed to two different plastic beads solution. As our research objectives of this part, we studied which plastic beads has stronger influence to the microorganisms. The target is to find the different impacts of the different charged microplastics on natural organisms. As we are located in Geneva around the Lake Geneva, in this step, the water condition of the Geneva Lake is also one of our research target. This study integrates both field work and laboratory experiment together. Firstly, samples from Lake Geneva will be collected with a manta net. Secondly, the content of plastic in these specimens will be isolated from the mixed materials at the laboratory in Versoix finally. In the last step of our study, we integrated our scientific research with the social theory of plastic impact on the natural environment. Related social discussion will be launched beside the scientific research. Being affected by this issue, the interdisciplinary discussion to the following areas were launched: the sources of this pollution, the impact on flora, fauna and human, the related legislation and policy making, the responsibilities of the different stakeholders, etc.

I hope this research can be a step to better understand and address the micro-plastic pollution problems in different aquatic systems. I expect this work will provide valuable insight into determining the origins of this pollution, the mechanism of the degradation, the influence to the environment, and that it will also offer a starting point for stakeholders, and society more broadly, to develop community-centered initiatives to reduce or even eliminate plastic pollution in our environment.

Abbreviations appear in this work: PS---PolyStyrene, PE--- polyethylene, PP--- polypropylene, PVC--- polyvinylchloride, HDPE--- high density polyethylene, LDPE--- low density polyethylene, PET---polyethylene terephthalate , PCB--- polychlorinated biphenyls, PAHs--- polycyclic aromatic hydrocarbons, DDT--- dichlorodiphenyltrichloroethane, PBDEs--- polybrominated diphenylethers, POPs--- Persistent Organic Pollutants, BPA--- bisphenol A, HCH--- Hexachlorocyclohexane, NP--- nano-particle, MP--- microplastic

1.1. General situation of the plastic pollution

Plastics are becoming necessary for modern life and, these properties of plastics also mean that plastics have become one of the most drastic impact factors on the environment. Over the past six decades, the rapid expansion of human activities has increased plastic production significantly, making the plastic materials a huge waste management problem (Olympic, 2013). It is estimated that plastic litter accounts for 10% of municipal waste mass worldwide and comprises 50–80% of the waste items stranded on beaches, floating on the ocean surface and lodged in the seabed finally (Barnes et al., 2009). Furthermore, it has been estimated that plastic materials make up to 60–80% of marine litter. Even though some plastic waste is recycled, up to 10% of plastics produced (by mass) is estimated to end up in the oceans where they accumulate (Barnes et al., 2009).

To the ocean, the major sources are runoff from land and the intentional dumping or loss of fishing and other plastic objects from ships, even though a complete ban on dumping of all forms of plastics into the sea was enacted in 1989 with the MARPOL regulations (Kummer, 1992). As we said before, once the macro plastics enter the ocean, they begin their slow degradation process, turning into the tiny micro-plastic fragments we found usually on beaches located thousands of kilometers from where they were initially discarded (Cooper and Corcoran, 2010a). A large proportion of this waste ends up in the sea and is transported all over the world by the oceanic currents circulation. This waste, being classified from macro-size to micro-size, has seriously endangered the aquatic systems, especially when they move up the food chain by “accumulation” for the aquatic fauna.

1.1.1. Quantity and distribution of plastics in aquatic systems

The production of synthetic plastics has increased dramatically since the 1950s. For example, among these plastics, Polystyrene is widely used as a packing material for food and consumer products (National Toxicology Program report, 2011). The global production of PS in 2008 reached 15.4 million tons, and around 20% of this arose from China (Feng et al., 2010). In addition, the Asia-Pacific region, including Korea, Japan, and China, has shown the most rapidly growing consumption of PS in the past decade (Saido et al., 2014). There are now 20 different main groups of plastics which have been produced (Halden, 2010), and their global production in 2011 was 280 million tons, of which approximately 150 million tons may still be in use, with the rest littering in continents and oceans (Rochman et al., 2013). Accumulation of plastic litter in marine and coastal environments has been appeared at the sea surface, on shorelines and the seafloor.

Up to now, it has been considered that most of the plastics, are resistant to chemical, physical, and biological degradation due to their molecular size and chemical stability (Halden, 2010; Cressey, 2011). The accumulation of plastics as the form of debris or fragments in the environment

received a substantial amount of coverage, and it is well known that these pollutants may persist for hundreds or even thousands of years (Weisman, 2008). Furthermore, until now, there are no exact estimates of plastic debris around the world ocean, and the environmental effects of plastics pollutants have been overlooked. Due to their resistance to physical, chemical and biological degradation, plastics are considered to be the stable material (Lithner et al., 2011) and are widely considered to not decompose at low temperature, through light or wave action. In this research area, there remains a significant absence of the information on the degradation of plastic debris and on the sediments of plastic debris in the ocean. The data on worldwide distribution are also incomplete.

As a result, plastics now contaminate every ocean of the world (Provencher et al., 2010). Large plastic debris, known as “macroplastics”, present an aesthetic problem for tourism (Jang et al., 2014), pose a risk to various marine industries (Sheavly and Register, 2007), threaten marine life through entanglement and ingestion, transport invasive species, and smother the seabed (Gregory, 2009). Microplastics, generally defined as plastics less than 5 mm diameter, are formed from the breakdown of macroplastics or sourced from the massive used in cosmetics and blasting media and are of increasing environmental concern now (Fendall and Sewell, 2009). Due to the durability of plastic and its persistence in marine environment (Sivan, 2011), microplastics have accumulated steadily since first being observed in the 1970s (Carpenter and Smith, 1972), and are now a kind of contaminant appeared all over the world’s oceans. According to the related research, the microplastics have accumulated in oceans different water column and sediments worldwide in recent years, with maximum concentrations reaching 100 000 particles per m³ (Wright et al., 2013). Due to their small size, microplastics may be ingested easily by low trophic fauna and influence the organisms.

Over the past decades, researches of the microplastics in the marine environment have increased. Microplastics consist of either manufactured commercial micro-particles, including some cosmetics, teeth pastes, and precursor pellets (primary sources), or as fragments and fibers derived from the breakdown of larger products (secondary sources; Hidalgo-Ruz et al., 2012).

A related study have been done on the measuring the relative abundance and mass of the plastic in the North Pacific Ocean in August of 1999 (See the Table 1). In this study, individual pieces of plastic were separated into different categories by type (fragment, Styrofoam fragment, thin plastic films) and their sizes. As the result showed finally, a total of 27698 small pieces of plastic weighing 424g were collected from the surface water at stations in the gyre, presenting a mean abundance of 334270 pieces /km² and a mean mass of 5114g/ km². The abundance ranged from 31982 pieces /km² to 969777 pieces /km², and mass ranged from 64 to 30169g/ km² (Moore et al., 2001).

TABLE 1Abundance (pieces km²) by type and size of plastic pieces and tar found in the North Pacific gyre.

Mesh-size (mm)	Fragments	Styrofoam pieces	Pellets	Polypropylene/ monofilament	Thin plastic films	Miscellaneous		
						Tar	Unidentified	Total
> 4.760	1931	84	36	16811	5322	217	350	24 764
4.759–2.800	4502	121	471	4839	9631	97	36	19 696
2.799–1.000	61 187	1593	12	9969	40 622	833	72	114 288
0.999–0.710	55 780	591	0	2933	26 273	278	48	85 903
0.709–0.500	45 196	567	12	1460	10 572	121	0	57 928
0.499–0.355	26 888	338	0	845	3222	169	229	31 692
Total	195 484	3295	531	36 857	95 642	1714	736	334 270

The quantities and types according to different size found in the North Pacific gyre (Moore et al., 2001)

1.1.2. Plastic pollution in the freshwater system

The microplastic pollution in marine water has attracted public and scientific interest in the last few years. However, the situation in freshwater environments remains unknown in a large scale, although they play an important role as part of the origin of marine plastic pollution. Apart from the physical impacts on biota, chemical effects are being expected as well, especially those caused by smaller particles. Recent studies have examined microplastics in lakeshore sediments (Zbyszewski and Corcoran, 2011), pelagic microplastics in rivers (Dubaish and Liebezeit, 2013) and lakes (Faure et al., 2012). Except Geneva Lake, similarly, surface water of the Laurentian Great Lakes (i.e, Lake Huron, Lake Superior, Lake Erie) was also sampled (Eriksen et al., 2013). Plastic particles have been analyzed in three different groups as different size. In a remote mountain Lake (Lake Hovsgol, Mongolia), an average value of 264 particles/km (435 particles/km²) was observed too (Free et al., 2014). It seems that all lakes are affected by the plastic pollution, microplastics of all types and composition having been found in all collected samples. The aquatic birds and fishes are easy to be affected by the microplastic ingestion, and the influences of the adsorbed pollutants (organic pollutants and heavy metals) surface of the microplastic should be also evaluated. Specification and quantification about their sources and the ecotoxicological impacts need to be done now. Other related questions, including the transport and fate of the plastic particles in the environment also should be studied (Faure and de Alencastro, 2014).

For example, a scientist Lechner used a stationary drift nets over a 2-year period (2010 to 2012) to estimate the plastic flow being conveyed by the Danube River (the Europe's second largest river) to the Black Sea at 4.2 tons a day (Lechner et al., 2014). Another related research have also been made on the Seine River (Gasperi et al., 2014). Many sampling sites are distributed along a 77 km stretch and their interception width varied between 5 and 15 m. Each site is cleared once a week using cleaner boats. Based on 6 years of monitoring (2008 to 2013), the total average mass of extracted floating debris amounts to 1937 tons (1591 to 2564 tons, SIAAP database (Parisian public sanitation service)). According to a related hypothesis, between 22 and 36 tons of plastics are intercepted annually by the sampling site, which are the major inputs of land-based sources. Such

data could contribute to a first evaluation of floating plastic inputs transported by rivers and released into oceans (Lithner et al., 2011).

In Switzerland, a related research about the plastic pollution in Lake Geneva has also been done by EPFL in Lausanne (Faure and de Alencastro, 2014). Their results showed the occurrence of such pollution in Lake Geneva. Further research has also been done by that team to identify the potential impacts and specify the situation for other Lakes in Switzerland, such as Lake Constance, Neuchâtel, Maggiore, Zurich and Brienz. Furthermore, the same sampling was done of the Rhone downstream Geneva, and the beach sediments have also been analyzed. The collected plastics have been sorted in different types (fragments, pellets, cosmetic beads, lines, fibers, films, foams). According to their report, related fishes and water birds have also been studied to assess their potential exposure and some other related analyses have also been conducted, such as the hydrophobic micro-pollutants adsorbed to the surface of the microplastics, some potentially toxic additives, etc. Their research results shows that all studied lakes are affected by this pollution, microplastics of all types having been found in all samples (See Annex 4; Faure and de Alencastro, 2014). Birds and fishes relying on these lakes are easy to microplastic ingestion, and all the tested chemicals (both adsorbed micropollutants and contained additives) were found with an high value. According to their further evaluation, in Switzerland, there are about 95% of the plastic either for recycling, direct energy recovery or incineration. The transfer to the environment is mostly by accidentally loss during transportation of waste, waste water, inappropriate waste storage or direct discharge into the environment by industries or individuals (Faure and de Alencastro, 2014).

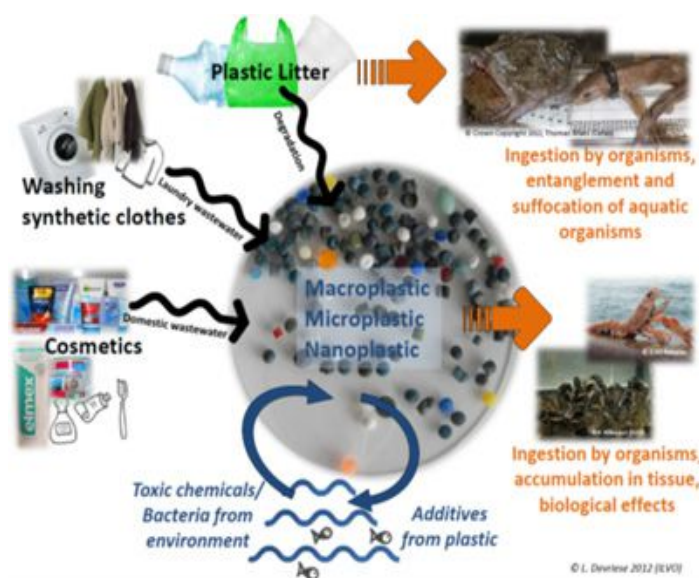
From this study, the microplastics are present on all the studied lakes, including beaches and surfaces with considerable quantities. The particle concentrations are often higher than those observed in the oceans. Microplastics are mainly from the fragmentation of larger objects, packages for a big part. However, there is a significant amount of foam, probably resulting from the construction sector. Pre-production of pellets, microbeads from cosmetics and other primary microplastic make up a small part of all. As those studies, the most frequently appeared particles were mainly PE, PP and PS.

1.1.3. The source of the plastic pollutant to aquatic system

The sources of marine microplastics are still not very well researched. A rough estimation predicts that 70% to 80% of marine litter, most of the plastics in this litter, originate from inland sources and are emitted from rivers to the oceans. Potential sources include wastewater treatment plants (WWTPs), beach litter, fishery, cargo shipping, and harbors (IOC, 2010). Although the exact data is unavailable, runoff from industrial plastic production sites may be an additional source as well. Taken together, most marine studies show that the inland waters is relevant main sources as

the important transport pathways. To the marine systems, major contributors of the microplastics include WWTPs and runoff from urban, agricultural, touristic, and industrial areas, as well as shipping activities. Another potential source is sewage sludge that typically contains more microplastics than effluents (Marine and Litter, n.d.). Sewage sludge is still frequently used for land filling and as fertilizer in agriculture, and surface runoff may transfer those microplastics to rivers and lakes and ultimately river basins and the sea. It is important to emphasize that the clothes washing and personal care products are the main sources of microplastics in WWTPs nowadays. For the limited capacity of wastewater treatment processes, a large quantities of microplastics released by WWTPs (Wagner et al., 2014).

As to the origin of microplastics, they come from a number of aquatic-based and land-based sources which can also be classified into two groups with the popular theory: primary microplastics (abrasive scrubbers, cosmetics, pre-production pellets, powder for air blasting) and secondary microplastics (fragments and fibers from larger plastic items; Hidalgo-Ruz et al., 2012). We can see the different origin of microplastic and their impacts on aquatic fauna from the Picture 1 (MICRO, 2014).



Picture 1: The origin and the impact of the plastic (MICRO, 2014)

The abundance and distribution of microplastics in the marine environment are governed by the surface circulation and winds, plastic density, shape, and distance to urban centers (Andrady, 2011). Microplastics may also form on land by UV degradation and fragmentation (Cole et al., 2011) or road abrasion of larger plastic items through damage by vehicles and transport along concrete pathways, but may also enter the aquatic environment through direct release. Polyethylene and polypropylene microbeads, used in many consumer facial cleansers, have been identified as potential contributors to marine microplastic pollution (Fendall and Sewell, 2009). Textile laundering facilities are also potential sources of microplastic fibers, and microplastic particles from sandblasting media have been suspected to pollute the marine environment since the early 1990s (Zitko and Hanlon, 1991). As related study, granulated polyethylene (PE), polypropylene (PP) or polystyrene (PS) particles, used for example in skin cleaners, can be introduced into wastewater. Furthermore, it has been shown that laundry washing machines discharge a large amount of plastic fibers into wastewater, with one study estimating that a single wash can produce 1,900 fibers (Browne et al., 2011). Industrial activities also contribute to the amount of microplastics in freshwater/aquatic ecosystems. High amounts of microplastic particles and fibers have been

detected near the industrial plants (Dubaish and Liebezeit, 2013). Synthetic fibers are also known to contaminate sewage sludges (Zubris and Richards, 2005).

It is clear that in order to answer this question more precisely, we need to consider the plastic lifecycle which involves processes that fall into two categories: (1) human production, consumption, and disposal of plastics, and (2) transportation and degradation of plastics once they enter natural systems. The production of plastics has been sharply increasing over the past 60 years. In 2012, over 300 million tons of plastics were produced worldwide, continuing the dramatical trend in plastic production from the 1950s, when only 1.4 million tons of plastics were produced (Rochman et al., 2013). This drastic growth in plastic production corresponds with our increasing dependence on plastics. Plastics have become extremely integrated into daily activities in both developing and developed countries. Our societies' use and disposal of plastics, in domestic and industrial settings, are the causes of all plastic pollution that ends up in natural systems, such as the system of oceanic currents. Once in the oceanic system, plastic pollution can then be transported around the world.

1.1.4. Plastic debris as the organic pollutants and heavy metals carrier

Aside from the physical influence to environment of plastic pollution, plastic debris can also act as a vector for transporting persistent, bio-accumulating and toxic substances (Frias et al., 2010; Koelmans et al., 2013; UNEP, 2011). For example, microplastics have been found to adsorb a wide-range of organic contaminants in the aquatic environment due to their hydrophobic nature. Contaminants that have been adsorbed include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenylethers (PBDEs), and bisphenol A(BPA) (Teuten et al., 2009). A researcher Rochman reported in 2013 that at least 78% of pollutants listed by the EPA (environment protection agency) and 61% listed by the EU adsorbed to plastic debris. Thus, the plastic pollution has been recognized as an important global environmental issue, and is becoming more and more severe.

Additionally, a study published by Katsuhiko in 2009 demonstrated that as drift plastic decomposes, it releases hazardous chemicals into the ocean. Polystyrene (PS) was found to begin decomposing at 30 °C, and to produce different toxic materials (Saido et al., 2009). This point raises another important element to keep in mind: plastic is not an inert material, and on top of its own toxicity it has the potential to agglomerate and transport Persistent Organic Pollutants (POPs). For example, following a related research, samples from beaches in South Africa have shown that polyethylene pellets concentrate and transport POPs in the forms of PCB, Hexachlorocyclohexane (HCH), and the pesticide such as DDT (Ryan et al., 2012), the same POPs have also been found on remote non-industrialized islands (Heskett et al., 2012). The surfaces of micro-plastics, which

contain toxic chemicals, are transported to remote areas and then these toxic chemicals are mostly released when ingested by organisms. This finding was highlighted in two perspectives on the global plastic pollution issue: the presence of plastic in some commonly consumed pelagic fish species and the plastic pollution continues to extend into the deep ocean through interconnected mesopelagic food webs (Choy and Drazen, 2013).

Regarding PCBs and PAHs, higher concentrations were observed in plastic fragments from urban beaches. Plastics are considered as important carrier of pollutants in marine environments. Because of the characteristics of PP and PE, they are lighter than water and persistent, they do not sink to coastal sediments unless entwined with other more dense debris and can be transported to the open ocean. It was proved from the related researches, in several plastic fragment samples from remote beaches and the open ocean, relatively high concentrations of additive-derived chemicals were detected as well (Hirai et al., 2011).

Along with persistent organic pollutants (POPs), heavy metals are another cause for concerning with plastic pollution. Just like POPs, heavy metals are attracted to plastic particles in the ocean, making the plastic much more toxic^{PAAD}. This then delivers a higher dose of toxins to any living beings unfortunate enough to mistake the plastic for food. Pollution by heavy metal ions, including mercury (Hg), lead (Pb) and copper (Cu), has become a major hazard issue due to their possible toxic effects (Leung et al., 2008). For example, in a related study, scientists established a novel and reliable method to estimate the total weight of beach litter and metal content of such litter material in Ookushi beach, Goto Island, Japan. Among the randomly collected beach litter, plastics composites 74% of the total weight, of which light plastics such as polyethylene were in big proportion. Among various toxic metals, lead (Pb) and total chromium (Cr) were detected in PE plastic litter. These heavy metals within polymers are often used in pigments such as lead chromate and are potentially released into the beach environment during the plastic degradation (Nakashima et al., 2012). These heavy metals bioaccumulate as their way up the food chain and by the time it gets to the top of the food chain (e.g, human beings).

As the special heavy metal adsorbing property, the plastic is even used to remove the heavy metals. Nowadays, among the various solid adsorbents, polymeric chelating resins are widely used in the removal of metal ions due to their high adsorption capacities (Vasconcelos et al., 2007; Masoumi and Ghaemy, 2013). As this adsorbents property, plastic are used in the adsorption of heavy metals because of their easy regeneration and strong mechanical properties in comparison with other adsorbents such as activated carbon, cellulose and silica gel. For example, one kind of plastic called CHA-111 AND MCH-111 polymeric adsorbents have been used as perfect adsorbents for adsorption pollutants such as phenols from waste water (Alsewailem and Aljlil, 2013).

PAAD: <http://plasticisadrug.blogspot.ch/2014/09/heavy-metal-loves-plastic.html>

1.1.5. Impact to aquatic organisms

The marine environment is one of the main hosts to increasing quantities of waste debris from human activities in and around the ocean. Any persistent or manufactured solid materials which are discarded in the marine or coastal environment is named marine debris, of which a large proportion consists of plastic (IOC, 2010). This debris is increasingly recognized as a threat to marine biota. For instance, by ingestion, about more than 267 species worldwide are estimated to be impacted by marine debris, including the majority of sea turtle species and almost 50% of all seabird and marine mammal species (Derraik, 2002). Many other studies are also concerned: scientist Laist (1997) lists 250 species in which constrictions or plastic ingestion causing choking or obstruction of the digestive tract were observed, ranging from whale to the fish, crustaceans and various birds. The most publicized cases are probably the Midway island albatross chicks die of starvation, stomach filled with plastics (Auman et al., 1997; See the Picture 2^{OCEANUS}) or fulmars in the North Atlantic over 90% of individuals found dead contained significant quantities of plastic (Avery-Gomm et al., 2012). The ocean now serves as one of the ultimate sinks for plastic waste as well as a host of other synthetic chemicals.

Picture 2: Sea bird death from the plastic consumption (OCEANUS)

In the ocean discarded plastic breaks into smaller plastic debris, which fragments into small-sized plastics that directly affect animal life and increase risks to human health in the end (Browne et al., 2008). They tend to break into smaller fragments as macro/meso/micro-plastics which have specific and significant set of impacts on ecosystem and can affect human and animal health negatively



associated with their chemical composition as well. Plastic fragments pose a significant hazard to many marine animals by entanglement, smothering, and ingestion (Avery-Gomm et al., 2012). Those plastic pellets and plastic fragments are frequently observed in marine environments nowadays. Since these materials have high decomposition temperature, high resistance to ultraviolet radiation and are not biodegradable easily. They can remain on both land and sea for years causing environmental pollution. Small plastic fragments are available to organisms at the base of the food web as they may be in the same size-range as natural food items. Related recent studies have shown that plankton and several classes of invertebrates and vertebrates can ingest and accumulate microplastics (Wright et al., 2013). Furthermore, the potential threats of the plastics to biota may not only the physical harm from ingestion, but also the bioaccumulative and toxic chemicals from the releasing of toxic additives, and desorption of persistent (IOC, 2010; GESAMP, 2010).

OCEANUS : <https://projectoceanus.wordpress.com/tag/ocean/>

The impact of such microplastics on wildlife remains poorly researched. The toxic effects, may derive from the original plastics but also from hydrophobic marine pollutants adsorbed onto the plastics from the surrounding water. Such pollutants are most likely to be ingested by deposit-feeding and filter-feeding organisms. With this way, those toxic pollutants are transferred through the food chain with the microplastics as the figure 1 demonstrated (Sutherland et al., 2010; Jennifer et Al., 2013). Furthermore, as their research, the presence of microplastics in Myctophid fish and

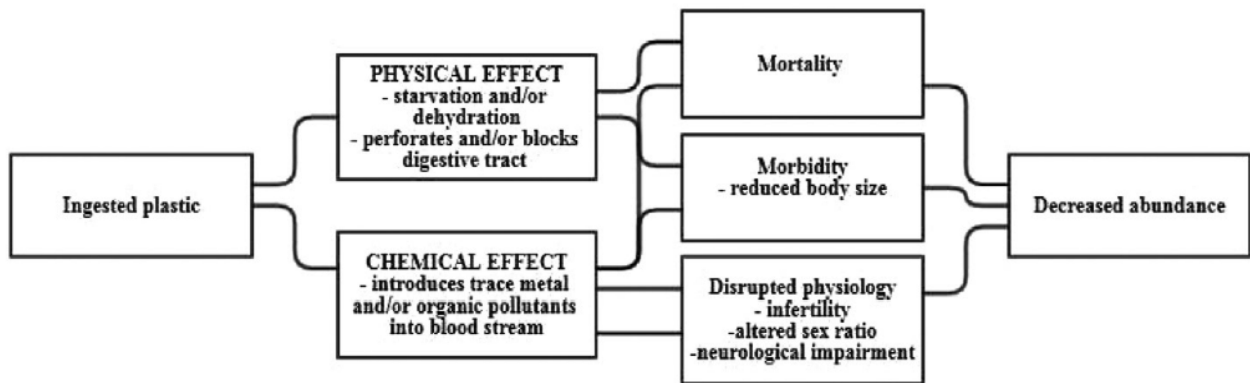


Figure 1: Potential pathways through which ingested plastic can affect marine wildlife (Jennifer et Al., 2013)

Hooker’s sea lion and fur seal scats proved that the pathway of the microplastic transferring through pelagic food chains: microplastics→zooplankton→myctophid fish→Hooker’s sea lions/fur seals. These trophic organisms present as a vector for microplastic transfer and their adsorbed contaminants.

There is no doubt that the ingestion of plastic debris has been shown to negative impact on seabirds in many ways, such as nutritional deprivation, reduced body mass, decreased fat deposition, and damage to or obstruction of the gut. Those ingested plastic has also been shown to influence the appetite and reduce growth rates of birds and other wildlife (Lavers et al., 2014). As we said before, plastic also attracts and accumulates organic pollutants (e.g., polychlorinated biphenyls, PCBs) and trace metals (e.g., cadmium, chromium, lead) (Etsuko Nakashima et al., 2012; Holmes et al., 2012; Lee et al., 2014). Once those plastic ingested, they may release the contaminants into the animal’s blood stream (Browne et al., 2013; Tanaka et al., 2013) which may result in stomach ulcerations, liver damage, neurological and reproductive effects, and death in the end (Lavers et al., 2014). Related researches show that the seabirds are the most heavily affected marine vertebrate with those plastic debris and are also declining faster than any other group of birds (Croxall et al., 2012). As the result, followed continuous few years of low breeding success, juvenile survival have been implicated in the decline of some marine species, including birds, likely the result of high mortality due to the ingestion of plastic (Priddel et al., 2006). Consequently, the ingestion of plastic debris by marine vertebrates is listed as a Key Threatening Processes under the Environment Protection and Biodiversity Conservation Act 1999 in Australia (DEWHA, 2009).

For the microplastic consumption, for example, with the Figure 2 showing (Todd Gouin et al 2011.), the filter feeders such as krill (which are the basic source of food for many marine organisms), forage fish, jellyfish, sharks, whales and sea birds are particularly affected by the ingested plastics due to their modes of food intake. As we showed before, plastic particles are some other chemical pollutant vectors that can be transported directly into the food chain. However, the quantification of transported mass fluxes is difficult to assess for the long range transport potential (LRTP) of chemicals. It is important to identify transport routes that plastic particles may directly serve as pollutant vectors into organisms and the food chain (Zarfl and Matthies, 2010).

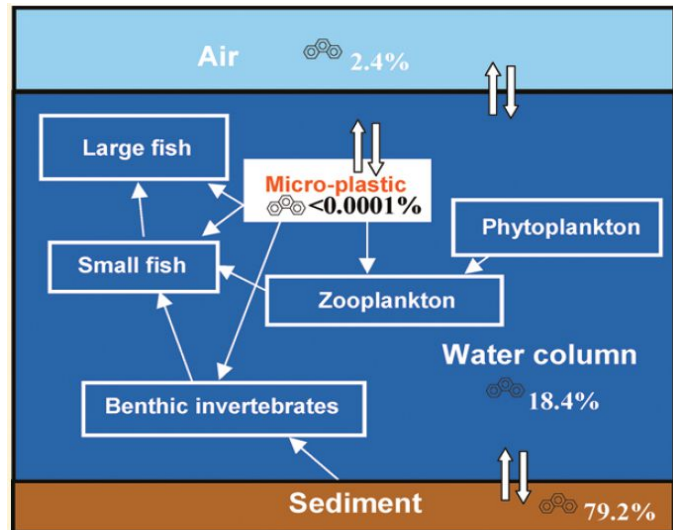


Figure 2: The microplastic in aquatic food web (Todd Gouin et al, 2011)

By the way, to be ingested by the aquatic fauna, the microplastics have to be bioavailable. There are four main factors which govern its bioavailability (Wright et al., 2013). **Firstly**, the key factor contributing to the bioavailability of microplastics is their small size, making them available to lower trophic organisms. Alternatively, higher trophic planktivores could passively ingest microplastics during normal feeding behavior or mistake particles for natural prey. **Secondly**, the density of the plastic particles in the water column will also determine their availability. Planktivores, filter feeders and suspension feeders inhabiting the upper water column are likely to encounter positively buoyant, low-density plastics, such as PE (specific gravity 0.91-0.94), on the sea surface. Alternatively, those high density microplastics could continue to sink, as the high density plastics such as PVC (specific gravity 1.38). Such particles will become available to benthic suspension and deposit feeders and detritivores as they sink, eventually reaching the benthos. **Thirdly**, the abundance of microplastics in the marine environment will affect its bioavailability as well, as the chance an organism will encounter a microplastic particle is enhanced. Therefore the progressive fragmentation of macroplastic items is likely to increase the amount of particles available for ingestion to a wider range of organisms. **Finally**, the color of microplastics may also potentially contribute to the possibility of ingestion, due to prey item resemblance. Some commercially important fish and their larvae are visual predators, preying on small zooplankton, and may feed on microplastics which most resemble their prey (Shaw and Day, 1994). Microplastics ingestion due to food resemblance may also be easily consumed by pelagic invertebrates, which are visual raptorial predators (Greene, 1985).

Despite the implementation of international legislation aimed at reducing the amount of marine debris originating from ocean and land-based sources, it continues to accumulate worldwide with a large quantity of new items entering the ocean each day. The number of marine mammals that die each year due to ingestion and entanglement reaches 1000 000 in the North Pacific Ocean alone (Wallace, 1985). Worldwide, 82 of 144 bird species have been examined that they contained small plastic debris in their stomachs, and in many species the incidence of ingestion exceeds 80% of the individuals (Ryan, 1990).

1.2. The plastic pollutant transformation mechanisms

As we introduced before, the microplastics come from two different ways. Firstly, the plastics that are manufactured to be of a microscopic size are defined as primary microplastics, such as some facial-cleansers and cosmetics, or as air-blasting media, some materials used in medicine as vectors for drugs. Secondly, the microplastics derived from the breakdown of larger plastic debris, both at sea and on land. Over time a culmination of physical, biological and chemical processes can reduce the structural integrity of plastic debris, resulting in fragmentation (Cole et al., 2011). Once plastics reach the aquatic environment, they fragment into smaller and smaller pieces following a number of mechanical, chemical and biological processes.

1.2.1. The degradation of the plastic debris

Microplastics are generally less than 5 mm in length, although size dimensions of microplastic classification can vary between research areas and research groups (Arthur et al., 2009). In the process of the plastic debris transporting along with the sea gyres, under the effects of thermal UV degradation and hydrolysis, photodegraded and oxidized plastic debris gradually becomes brittle. While in the sea, plastics begin to break down by either Picture–, thermal, or biological degradation. Large, low density plastic fragments floating on the surface of the ocean become brittle and break into smaller pieces while exposed to UV radiation, oxygen and seawater (Andrady, 2005). However, plastic debris on land degrades more easily than plastic at sea because of higher solar radiation exposure and subsequent increased temperature (Pegram and Andrady, 1989). Due to chemical weathering and mechanical processes, the plastic debris are transformed or degraded into microplastic (Cooper and Corcoran, 2010). As to the different size, density and abundance of the microplastics, they distribute in different layers of the aquatic system, and furthermore, ingested by different fauna which situated at the different layers as Figure 3 shows below (Wright et al., 2013).

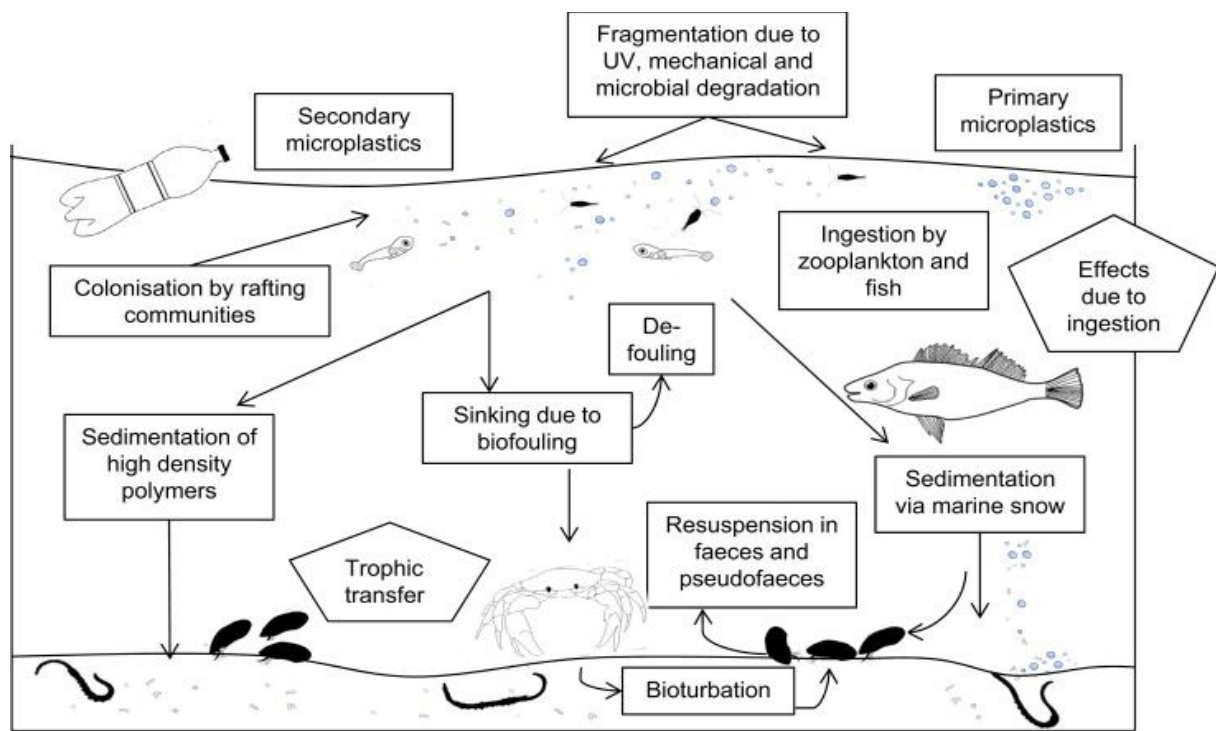


Figure 3: Potential pathways for the transport of microplastics and its biological interaction (Wright et al., 2013)

For understanding the plastic debris degradation, density measurements is one of the important parameters. It can be a powerful aid in characterizing the plastic types present in the ocean. They also demonstrate that chemical and physical particle properties change at sea, likely due to microbial biofouling. Differences in density distributions of beach and pelagic plastic debris provide critical data for understanding debris sources, transport, and fate. The utility of these data emphasize the need for future studies to address the factors that change plastic densities in differing marine environments. Furthermore, studying the size, mass, and composition of plastics that persist in the surface ocean is important as well to understand the impacts of plastics on seabirds and to identify and mitigate the sources of this debris (Morét-Ferguson et al., 2010).

For the density modification of pelagic plastic particles, two possible hypotheses are proposed from the related researches. One is weathering, photochemical breakdown and prolonged mechanical abrasion which easily alter particle-density with exposure. However, it is currently not clear if weathering can lead to increases in density. Another is biomass accumulation, often visually observed on the samples, altering its density as well (Morét-Ferguson et al., 2010). So, little is known about the exactly rate and mechanisms of plastic degradation and fragmentation in the freshwater environment. However, plastics degrade more quickly when dried and exposed on land than when in the water, and the pace of plastic degradation may be driven more by terrestrial processes. But it may not vary in big differences between freshwater and marine shores (Free et al., 2014). So, still more researches are required to understand the rates and mechanisms of plastic degradation in freshwater and the role of these processes in determining microplastic density and also for their distribution.

1. 2.2. The plastic characteristics transformation by their densities

As we said before, the density of the plastic particles is one of the essential factors to influence the plastic particles bioavailability in the water column and also maybe influence the toxicity of microplastics to the aquatic organisms. An example from related research, the planktivores, filter feeders and suspension feeders situated at the upper water column are more likely to encounter positively buoyant, low-density plastics, such as PE, on the sea surface. The buoyancy of plastic is influenced by biofouling. For example, the PE food bags cultivate a well-developed biofilm within one week, which continued to increase with a three week exposure period. After the third week, these bags start to sink below the sea surface (Lobelle and Cunliffe, 2011). According to this research, the rate of biofouling depends on some necessary parameters, such as surface energy and hardness of the polymer, as well as water conditions. However, for microplastics, it also can be return to the sea-air interface by some special organisms during their predating. This pattern may make microplastics available to organisms living in different depths in the different water column. The fouled microplastics could continue to sink with high density plastics such as PVC (specific gravity 1.38). Such particles will finally become available to benthic suspension and deposit feeders and detritivores when they reach the benthos (Lobelle and Cunliffe, 2011).

1.3. Physico-chemical properties of nano sized latex particles

When the macro-plastics are transformed into microplastics and to the nano sized particles in the end, those nano sized particles suspend in different aquatic colloids groups with different biophysical and chemical factors. As the result, the properties and the stability of the colloids highly influence the existing conditions of microplastics and their bioavailability to aquatic organisms.

1. 3.1 General consideration on colloid stability in aquatic systems

As the research of Professor Jacques Buffle in Geneva University, in aquatic systems, through covalent, electrostatic, or hydrophobic interactions, a large proportion (often 40-90%) of trace compounds may be adsorbed on marine and freshwater colloids. Consequently, the properties and behavior of the sub-micron colloids will play key roles in the fate of trace compounds during the colloids. When they are stable in solution, they may be transported for long distance. However, the function of coagulation or flocculation may facilitate colloidal elimination through sedimentation (see Figure 4). In complex systems such as natural waters, colloid aggregation can be seen everywhere due to the large number of colloid types and reactive sites. Indeed, size fraction analysis of natural aquatic colloids often demonstrates a physicochemical uniformity among all fractions as Figure 4 (Buffle et al., 1998).

For the particles and the colloid groups, adsorption, complexation, aggregation and sedimentation processes in ecosystems are very important for interpreting and predicting their reactivities. The transport and fate of both nutrients and toxic compounds mostly depend on their interactions with colloidal particles and biopolymers, the complexes and aggregates, their form and settling velocities. The circulation and elimination of their compounds then depends upon the kinetics of formation, structure, and sedimentation rate of these aggregates. Because of the complexity and large number of biophysical and chemical factors influencing these processes, there is no unique theory which can directly be applied to all these dynamic processes in aquatic systems (Stoll et al., 2009).

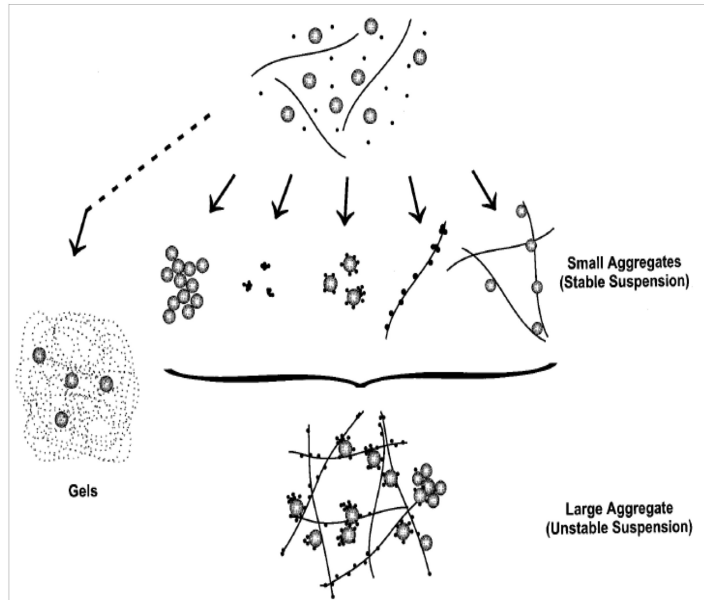


Figure 4: Major aggregates types formed in the three-colloidal component system (Buffle et al, 1998)

1.3.2. Latex particle coagulation and sedimentation

From the Figure 5, in certain circumstances, the particles in dispersion may adhere to one another and form aggregates of successively increasing size. They may settle out with the influence of gravity. From the related study, an initially formed aggregate is called a floc and the process of its formation is called flocculation. The floc may or may not sediment or phase separate. It depends on their properties in this certain situation. If the aggregate changes to a much denser form, it is encountering coagulation. An aggregate usually separates out either by sedimentation (if it is more dense than the medium) or by creaming (if it less dense than the medium). Usually coagulation is irreversible whereas flocculation can be reversed by the process of deflocculation^{ZETA}. The Figure 5 shows the various mechanisms where the colloids stability may be lost in a related colloidal dispersion.

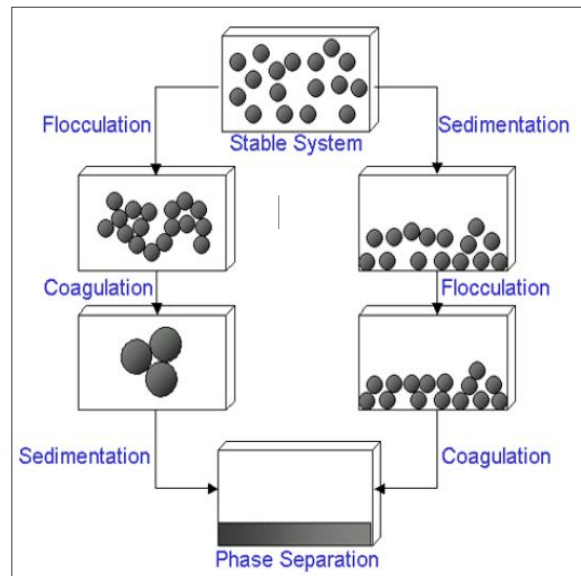
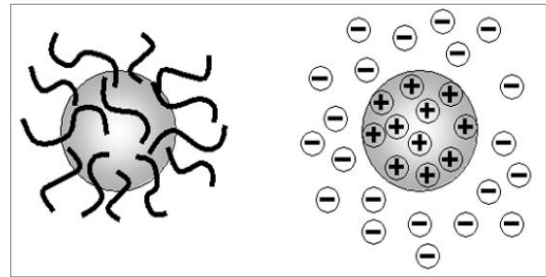


Figure 5: Schematic diagram showing various mechanisms where stability may be lost in a colloidal dispersion (ZETA)

ZETA: <http://www3.nd.edu/~rroeder/ame60647/slides/zeta.pdf>

Therefore to maintain the stability of the colloidal system, the repulsive forces must be dominant. How can colloidal stability be achieved? There are two fundamental mechanisms that affect dispersion stability: Steric repulsion and electrostatic stabilization. The Picture 3 shows the Steric and electrostatic stabilization mechanisms of colloidal dispersions.



Picture 3: Steric and electrostatic stabilization mechanisms of colloidal dispersions (ZETA)

The Steric repulsion, it involves the polymers being added to the system and adsorbing onto the particle surface and preventing the particle surfaces coming into closer. Imagining if enough polymer adsorbed, the thickness of the coating will be sufficient to keep particles separated by steric repulsions between the polymer layers. The Electrostatic or charge stabilization is the effect on particle interaction due to the distribution of charged species in the system. Each mechanism has its benefits for particular systems. Steric stabilization is simple, requiring just the addition of a suitable polymer. However it may be difficult to flocculate the system if this is required, the polymer can be expensive and in some cases the ideal polymer is undesirable. Electrostatic or charge stabilization stabilize or flocculate a system by altering the concentration of ions in the system. This is a reversible process and is potentially inexpensive. It has been recognized that the zeta potential is a very good index of the magnitude of the interaction between colloidal particles. The measurements of zeta potential are commonly used to assess the stability of colloidal systems for this reason^{ZETA}.

2. Experiment goals and procedure

In our study, we initially focused on a broad bibliography which address the general issue of plastic pollution in the aquatic environment, the types of plastics in the environment, plastic transformation mechanisms, sources of plastics and the impacts of these pollutants to the environment.

Secondly, a laboratory experimental study of plastic beads was conducted to assess the behavior of these latexes in Milli-Q water, culture media and natural water. On those basis, thirdly, the microplastic ecotoxicity essay also has been done with the zooplankton (*Daphnia magna*).

At same time, samples were collected in Lake Geneva to analyze the plastic pollutants quantity, types and their possible sources. These

samples then were isolated and analyzed with a serial of processes and the different types of plastics were collected in the end. Finally, the impact of the plastic pollutants in our natural aquatic systems has been analyzed by integrating all those research objectives. Figure 6 shows us schematically these experiment goals and procedures.

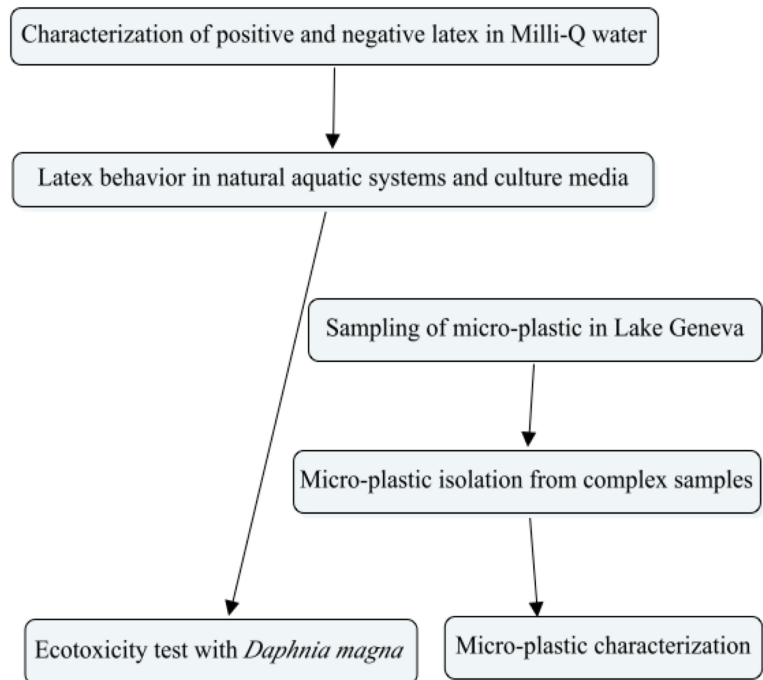


Figure 6: Schema of my experiment goals and procedure

3. Materials, methods and instrument

3.1 Materials

The selected microplastic materials are spherical particles in the colloidal size range that are formed from an amorphous polymer such as polystyrene. Because of the way that the polystyrene chains arrange themselves in the bead, the surface is very hydrophobic in character, making these ideal materials for the adsorption of materials such as proteins. (For well maintain it's properties, all these micro-beads should be stored in deionized water with the temperature between 2-8 °C.)

a) Amidine Latex Beads, 4% w/v, 0.2 µm: This positively-charged hydrophobic Amidine latex is particularly suited for the preparation of latex intermediates. The only surface functional group present on the particle is Amidine, which is not sensitive to the aggregating effects of polyvalent cations. These particles are sensitive to negatively charged contaminants or multivalent anions. The Amidine latexes should be used in low to neutral pH environments. The Amidine group is a base and with a pK ~10-11.

This positively charged polystyrene microspheres is covered with Amidine functional groups on the surface. The surface charge is pH dependent (stable with low pH). It's surface is hydrophobic in nature. Normally, the particle Number is equal to 7.3×10^{12} /ml and the surface charge density reaches about $13.2 \mu\text{C}/\text{cm}^2$. The charged groups per particle are about 1.2×10^5 . For more information, see Annex 1^{Amidine}.

b) Carboxyl Latex Beads, 4% w/v, 0.2 µm: The carboxyl latex consists of carboxyl charge-stabilized hydrophobic polystyrene microspheres. Only carboxyl groups are present on the particle surface. The particles are the carboxyl analog of the sulfate latexes. The pKa of the carboxyl group is relatively high, and consequently these particles are not suitable for work in acidic media. Carboxyl latexes are available in a range of sizes and surface charge densities.

The Carboxyl latexes should be used in neutral to high pH environments. This negatively charged polystyrene microspheres is covered with Carboxyl functional groups on the surface. The surface charge is pH dependent and is stable with high pH which is different from the Amidine latex. It's surface is hydrophobic in nature. Normally, the particle number is 8.0×10^{12} /ml and the surface charge density reaches about $10.3 \mu\text{C}/\text{cm}^2$. The charge groups per particle are 8.9×10^4 . For more information, see annex 2^{Carboxyl}.

Amidine: https://tools.thermofisher.com/content/sfs/COAPDFs/2014/1627736_A37314.pdf

Carboxyl: https://tools.thermofisher.com/content/sfs/COAPDFs/2015/1704377_C37486.pdf

3.2 Methods and Instruments

a) Zetasizer (from Malvern)(Picture 4^{Zetasizer}): The Zetasizer was used to measure the behaviors of the microplastics: a) in ultra-pure water; b) in natural water; c) *D.magna* growing medium. The Zetasizer Nano Z is a system where the highest sensitivity and widest size range (0.0038 μm to 100 μm) is required. It is a particle size analyzer for the detection of aggregates, measurement of small or dilute samples, or samples at high concentration. It is also ideal for the molecular weight analyzer, molecular size, zeta potential and molecular size measurement. It integrates two techniques (Dynamic Light Scattering, Static Light Scattering) in a single compact unit, and has a range of options and accessories to optimize and simplify the different measurement (Figure 7).

From the Malvern instrument introduction, Dynamic Light Scattering is used to measure particle size and molecule size. This technique measures the diffusion of particles moving under Brownian motion, and converts this to size and size distribution by using Stokes-Einstein relationship. Static Light Scattering is used to determine the molecular weight of proteins and polymers. In this technique, the scattering intensity of a number of concentrations of the sample is measured, and used to construct a Debye plot. From this the average molecular weight, it can give a measure of solubility.

What is the zeta potential? Zeta potential is a physical property which is showed by any particle in suspension, macromolecule or material surface. It is used to optimize the formulations of suspensions, emulsions and protein solutions. It is also used to predict surfaces interactions and optimize the formation of films and coatings. As related research, the liquid layer surrounding the particle exists as two parts: an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move it. Those ions beyond the boundary stay with the bulk

Picture 4: Zetasizer Nano Series
(Malvern: Zetasizer Nano Series User Manual, 2004)

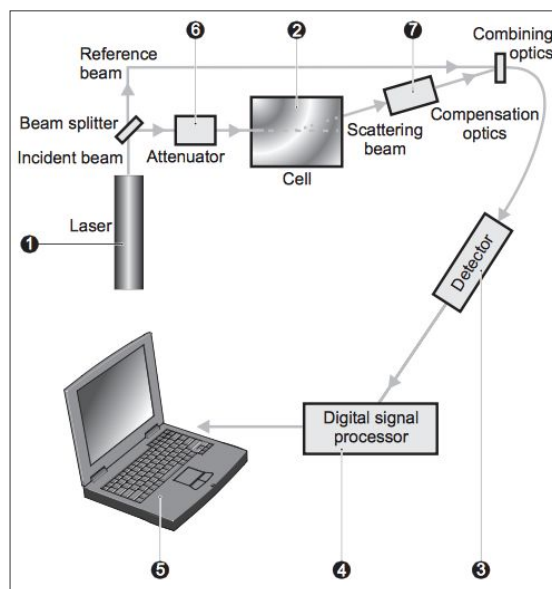
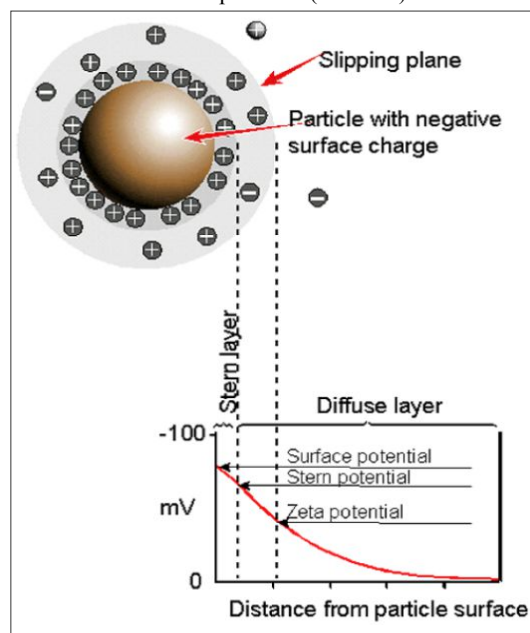


Figure 7: Optical configuration of the Zetasizer Nano Series for the zeta potential measurements (Malvern, 2004)

dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential (Figure 8^{Malvern}).

The magnitude of the zeta potential gives us an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable (Figure 12). However, if the particles have a density greater than the dispersant, even though they are dispersed, they will eventually sediment forming a close packed bed.

Figure 8: Schematic representation of zeta potential (Malvern)



So, the physical properties of colloids and suspensions are strongly dependent on the nature and extent of the particle-liquid interface; the behavior of aqueous dispersions being especially sensitive to the electrical and ionic structure of the interface. The production and stability of colloids and suspensions are both intimately related to the so-called electrical double layer that characterizes the interface. Information relating to stability is therefore of considerable importance. It should be noted that the term stability, when applied to colloidal dispersions, is generally relative in meaning and intended to express the resistance to change of the dispersion with time. Zeta potential measurements are directly related to the nature and structure of the electric double layer at the particle-liquid interface. Zeta Potential is an important parameter for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle. Zeta Potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). It has been recognized that the zeta potential is a very good index of the magnitude of the interaction between colloidal particles and measurements of zeta potential are commonly used to assess the stability of colloidal systems. The Zetasizer Nano Z is the perfect system for measuring zeta potential and electrophoretic mobility of colloids and nanoparticles.

So we can say that the zeta potential is a key indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between

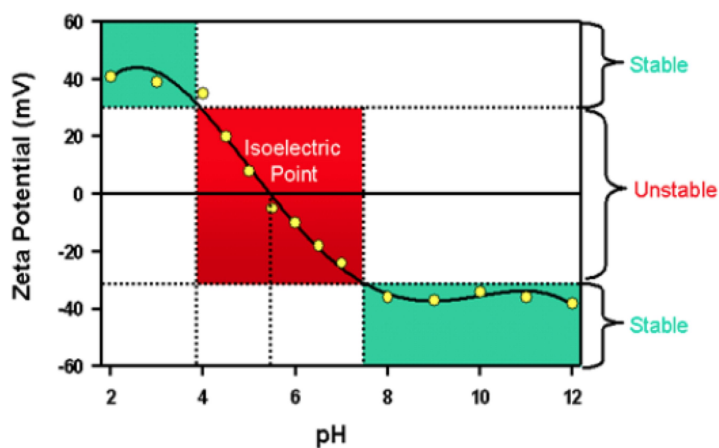
Malvern: Zeta potential - An introduction in 30 minutes. ht

<http://www.malvern.com/en/support/resource-center/technical-notes/TN101104ZetaPotentialIntroduction.aspx>

adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is small, attractive forces may exceed this repulsion and the dispersion may break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate.

In aqueous media, the pH of the sample is also one of the most important factors that affects its zeta potential. Imagine a particle in suspension with a negative zeta potential. If more alkali is added to this suspension then the particles tend to acquire more negative charge. If acid is added to this suspension then a point will be reached where the charge will be neutralized. Further addition of acid may cause a building up of positive charge if the ions are specifically adsorbed. In this case a zeta potential versus pH curve will be positive at low pH and lower or negative at high pH. The point where the plot passes through zero zeta potential is called the isoelectric point and is very important from a practical consideration. It is normally the point where aggregation is most likely and hence the colloidal system is least stable.

Figure 9: Typical plot of zeta potential versus pH showing the position of the isoelectric point and the pH values where the dispersion would be expected to be stable (Malvern)



A typical plot of zeta potential versus pH is shown in Figure 9^{Malvern}. In this example, the isoelectric point of the sample is at approximately pH 5.5. In addition, the plot can be used to predict that the sample should be stable at pH values less than 4 (sufficient positive charge is present) and greater than pH 7.5 (sufficient negative charge is present). Problems with dispersion stability would be expected at pH values between 4 and 7.5 as the zeta potential values are between +30 and -30mV .

b) Standard Grade Regenerated Cellulose (Spectra/Pro Membranes MWCO) (Picture 5) : It is a clear, flexible and sturdy dialysis membrane with a certain molecular selectivity. This economical membrane is ideal for a broad range of applications in which there is a significant size difference between the MW species being separated; including sample prep, desalting, buffer exchange, pH change, protein purification, etc. Offering a good chemical compatibility, it can be used with dilute strong acids and

Picture 5: The membrane measurement instrument designed by our research group



Malvern: Zeta potential - An introduction in 30 minutes. ht
<http://www.malvern.com/en/support/resource-center/technical-notes/TN101104ZetaPotentialIntroduction.aspx>

bases, concentrated weak acids and bases, most alcohols and some mild or dilute organics, including DMSO. Standard RC can tolerate pH 2 - 12 and temperatures 4-121 °C.

The target of our experiment is trying to measure the modification of the latex properties in natural aquatic systems. We need the latex to be in contact with the milieu but avoiding loss. The **Standard Grade Regenerated Cellulose** is one kind of ideal material to meet our purpose. It can not only let the natural water enter the membrane to contact the latex freely, but keep the latex stay in the membrane safely and avoid the plastic latex polluting the natural aquatic systems.

c) Instrument for the latex behavior measurement during a residence time in natural water

Amidine and Carboxyl were put in the Versoix river and Geneva lake with a specially designed instrument during 6 days. Firstly, we filtered the water from Versoix river and the Geneva lake water with a filter of 0.4mm for my experiment use. The photos below with different colors show the water characteristics somehow. In Versoix river, there are more different SPM (suspended particle matter) than Geneva lake. The color indicated the differences of the materials between these two different water systems (Picture 6).



Picture 6: Physical differences between the lake water and river water

In order to create a real natural condition between our latex solution and the natural water, we set up the instruments like the Picture shows below (Picture 7). The Amidine solutions of 100mg/L were put in the membrane (**Spectra/Por Membranes MWCO, 45mm broad**) before put in the Versoix river and Geneva lake (two instruments in each natural aquatic condition). The membrane is a ideal material for our experiment purpose. It allows the natural water enter the membrane to contact the latex freely(the expected biofilm developed totally), but keeps the latex stay in the membrane safely and avoid the plastic latex polluting the natural aquatic systems. (pH: river 8.34, lake 8.07; conductivity: river 374us/cm, lake 285us/cm)

With 6 days' residence time in the Versoix river and the Geneva lake, the two latex solutions were collected carefully. Fortunately, the solutions were well developed except one of the membranes in the Geneva lake was broken.



Picture 7: Latexes in membrane putted in Versoix river and Geneva lake

d) fluorometer (Picture 8^{Fluoromete}) : In our experiment, the value of the Colored Dissolved Organic Matter (CDOM) is one of the important parameters in modifying the surface properties of the latex. CDOM will also fluoresce after light absorption allowing researchers a way to detect and quantify its abundance in water systems using fluorometry. As this reason, the value of the CDOM is very important to explain the change of the latex properties in different CDOM contained milieus. The fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. These parameters are used to identify the presence and the amount of specific molecules in a medium. In our experiment, it is used to calculate the quantity of the Dissolved Organic Matter in a given solution.



Picture 8: Fluorometer (Tuner Design)

The Dissolved Organic Material (CDOM) exists in many forms ranging from naturally occurring humic acids to by-products or secretions excreted from organisms. CDOM is a highly abundant form of organic matter and represents a major reservoir of reactive carbon. It is also a dynamic substrate which can undergo reactions to become accessible to bacteria, plants, and animals as an energy source or it can photodegrade resulting in the production of volatile compounds that can have adverse effects on organisms and the environment. CDOM typically contain chromophores that absorb UV and visible light, hence the term Chromophoric (or colored) Dissolved Organic Materials (CDOM) (Tuner Design, Fluorometer).

e) Sanyo Incubator Mir-253 Manual (Picture 9^{SANYO}): SANYO's MIR series incubators have been recognized as exceptional units suitable for a wide range of applications by accommodating a temperature range of from -10°C to 50°C . In pursuit of temperature precision and enhanced operability, the new MIR-153/253/553 series makes its debut. Incorporating an 8-bit microcomputer, these incubators control the heater and compressor within a precise ± 0.2 deg. and

Fluoromete: <http://www.turnerdesigns.com/applications/dom-fluorometer-application-notes>

SANYO: <http://www.geminibv.nl/labware/sanyo-koelbroedstoof-mir-153/sanyo-mir153-553-manual.pdf>

± 1 deg. range, respectively. In addition, they can be applied to a wide variety of experimentation patterns with the aid of a 3-step microcomputer program. These cooled incubators are designed to meet a variety of advanced experimental needs ranging from microorganism cultures and plant germination tests to various constant temperature experiments. Combining flexible Temperature (T) and Time (H) control, a maximum 3-step plus constant operation or max 3-step repeating operation can be programmed according to the experimentation requirements. The one-step setting time ranges from 0.0 to 99.5 hours in increments of a half hour. A program can be set to repeat for a minimum of once up to a maximum of 99 times. This incubator accommodates a range of diversified experimentation requirements, and is ideal for experimentation during night time or holidays, experimentation that requires settings to be changed, and microorganism culture and preservation. Constant operation mode without step operation is also available. It was used to hatch the *Daphnia magna* dormant eggs for our ecotoxicity essay (Sanyo Incubator Mie-253 Manual).



Picture 9: Sanyo Incubator Mie-253 (SANYO)

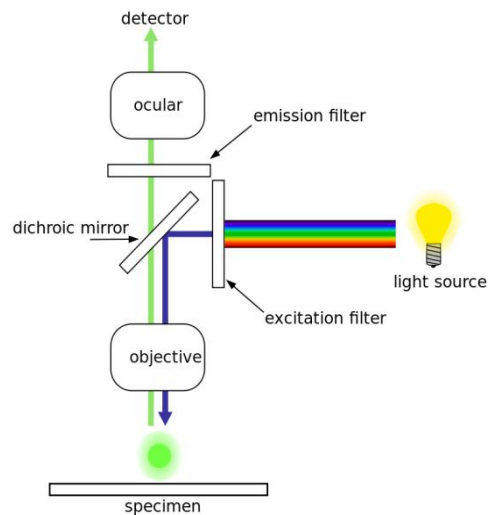
f) Fluorescence microscope (OLYMPUS BX61, MODEL: BX-UCB) (Picture 10, Spring KR et al, 2008) The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image.

Picture 10: Fluorescence microscope (Spring KR et al, 2008)



The specimen is illuminated with light of a specific wavelength which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter (Picture 11, Spring KR et al, 2008). In this manner, the distribution of a single fluorophore (color) is measured and imaged at a time. Multi-color images of several types of fluorophores must be composed by combining several single-color images. Precisely, Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greatest sensitivity will have a very high numerical aperture.

For the specimen to be viewed sufficiently, image brightness is a key and needs to be achieved using the correct wavelength of light. Selecting an efficient barrier filter (available from manufacturers) helps to allow for selected wavelengths of light, those that excite specific fluorophores, to enter the observation eyepiece while correctly blocking others. It brings the traditional optical microscopy into the nano-dimension. In our experiment, the consumption of the plastic latex by *D.magna* is clearly illustrated by those related images. The fluorescence microscope is very important to visualizing the ecotoxicity of the plastic latex to the aquatic organism (Spring KR et al, 2008).



Picture 11: Schematic of a fluorescence microscope (Spring KR et al, 2008)

g) Methods of the bioassays with *Daphnia magna*:

Daphnia magna (Picture 12^{MBLA}): *Daphnia magna* is crustacean zooplankton measuring from 1 to 5 mm, the family of the genus *D.magna* water fleas Phyllopod. They live in fresh water, stagnant and some species tolerate slightly brackish conditions. They have a transparent body, squat and drop-shaped asymmetric water, protected by a translucent shell. It has large feathery antennae, used for swimming. To feed, *D.magna* filters the water and capture tiny planktonic organisms. *Chronic Daphtoxkit* test is based on measuring the degree of immobilization of *Daphnia magna* in contact with the tested pollutant. We use the immobilization of the *D.magna* in the aimed pollutant solution for evaluating toxicity for this test.

Picture 12: *Daphnia Magna* (MBL Aquaculture)



The use of *D.magna* as an experimental organism for such purposes is advantageous in many respects. *D.magna* are small, reaching a size of five mm, so that a great many can be reared in a small space. They have a relatively short life span, which reaches a maximum of about two months when they are reared at 20°C. *D.magna* are easy to culture, requiring only water containing bacteria or their equivalent for food. They can be grown individually in small bottles or in mass culture in large aquaria. They mature early, giving birth to young within their first week of life. After their first brood, they give rise to new broods every two or three days throughout the remainder of their lives. An average of twenty or more young may be produced in each brood. Each female who lives to a ripe old age can bear four hundred or more offspring. Again, all the young from one female are genetically like the mother if produced parthenogenically, and reproduction can be limited to

MBLA: MBL Aquaculture, <http://mblaquaculture.com/content/organisms/daphnids.php>

parthenogenesis if the proper conditions are maintained. Further, *D.magna* are representatives of a class of animals that serve as food for many fish, especially while the fish are young. Fish do not remain in water where their food supply has been depleted. *D.magna* would be affected if there was something toxic added to the water, therefore fish would leave and the *D.magna* would die^{Wiki-D.m}. Furthermore, the size of the *D.magna* is perfect for our plastic latex toxicity essay. For all these reasons *D.magna* proves satisfactory for our testing on.

Method of the bioassay: To perform the test with *D.magna* (from the kit marketed Daphtoxkit), the eggs must first be incubated (Sanyo Incubator Mir-253 Manual as we explained before) for 72 h at 20-22 ° C under continuous illumination 6,000 lux. Ecotoxicological tests using newly hatched *D.magna* and then must be strictly conducted within 24 h and fed two hours before the test.

Exposures are performed using plates in which *D.magna* are exposed to several concentrations of pollutant as the **Figure 10** shows. Thus, the exposure solutions therefore were first prepared, the test plates filled, and then the organisms added. After 48h, the number of immobilized organisms in each cell was recorded.

In our experiments, firstly, two different latex solutions (Amidine and Carboxyl) were prepared in concentrations (100 mg/L, 50 mg/L, 20 mg/L, 5 mg/L, 0.5 mg/L, 0 mg/L (control)). Secondly, the cells of the plates were filled 10ml of each latex solutions(10ml for each cell). 25 *D.magna* organisms were carefully transferred to the first cell of each line and then the organisms will be distributed to other four cells (5 in each cell). Finally, the plates containing organisms and latex solution were put in an incubator and the immobile *D.magna* numbers will be counted after 48h. The same experiment was explored 3 times and the average EC50 (*D.magna* EC50, wiki) were got according to the immobilized *D.magna* number.

With the three times experiments and the average results of immobilized *D.magna* number in each corresponding latex solutions concentration, a curve of *D.magna* immobilization and latex concentrations was constructed. This ecotoxicological test will allow us to determine different levels of toxicity induced by the plastic latex on an organism (*D.magna*). EC50 means that the most commonly used toxicity levels of the concentration causing adverse effects in 50% of organisms. According to the curve we made, we choose the value of 50 on *D.magna*

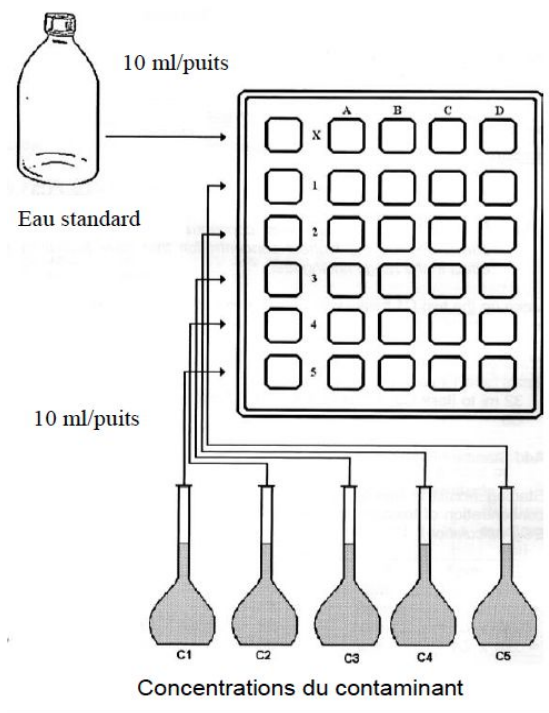
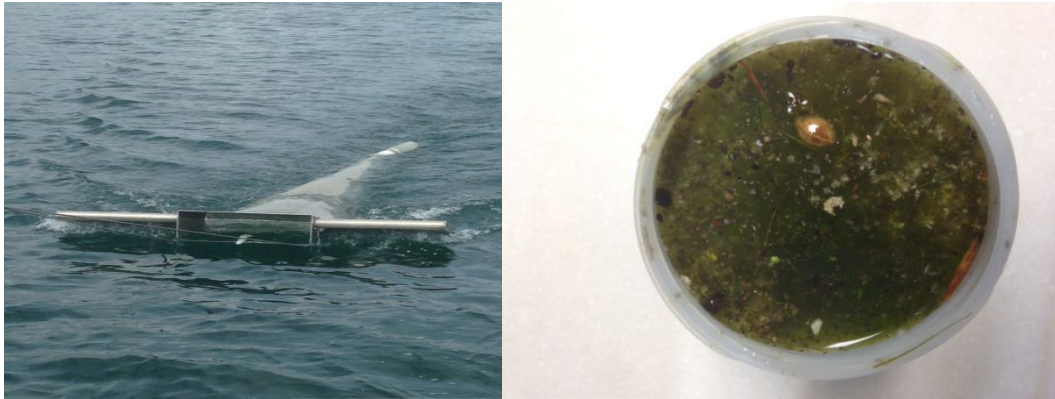


Figure 10: Schematic of the toxicity experiment (Modules d'enseignements thématiques, MUSE, unige, 2014)

Wiki-D.m: https://en.wikipedia.org/wiki/Daphnia_magna

immobilization curve. According to the immobilization point on the curve, we can situate the corresponding latex concentration this concentration is just the wanted EC50.

h) Manta net (Picture 13): Sampling the lake surface was performed with this manta net (Picture 13) which is the most commonly used instrument in such studies. It's a 300 microns (300 μm) mesh with an opening of 60 cm broad and 18cm wide. The net floats at the water surface and kept stable by the two strings. It maintained on the side of the boat with a pole or a telescopic arm at a distance of about 3 meters to avoid waves caused by the boat (wave action).



Picture 13: Manta net and the filtered samples

i) Sampling: The sampling was launched on 15/06/2015 and we filtered a distance of about 3 km at each sampling point. The trajectory of boat is recorded by GPS, and the actual filtered water quantity was measured by a mechanical flow meter attached to the opening of the net. Four samples sites (2 in the small-lake sites and 2 in the big-lake sites) (the sampling site started coordinate GPS: site1 GPS: 503777,127653; site 2 GPS: 528818,142458; site 3 GPS: 533644,145299; site4 GPS: 510075,133925) were collected from the surface of the Geneva Lake according to their different locations. At each site, the samples were collected with the ship speed of 6 km/h during 30mins (It means we filtered about 324m³ at each sampling site). Sampling is done in light winds to prevent the particles undergo vertical mixing in the water column, and to keep the net stable and immersed at the surface to ensure a constant filter volume. Boat speed remains constantly around 6 km/h for the same reason. When the filtering is done, the net was filled with lake water to push the mixed material at the bottom of the net. We carefully then turned over the net and washed the material until it being totally collected in the buckets.

j) Filtering: After collection from the lake, samples were stored in buckets (PP) 4°C in salt saturated brine (adding 250g of NaCl per liter to preserve the filtered complex materials and increase the water density at same time) until analysis. Materials from each samples was then separated by gravity and collected. In further step,



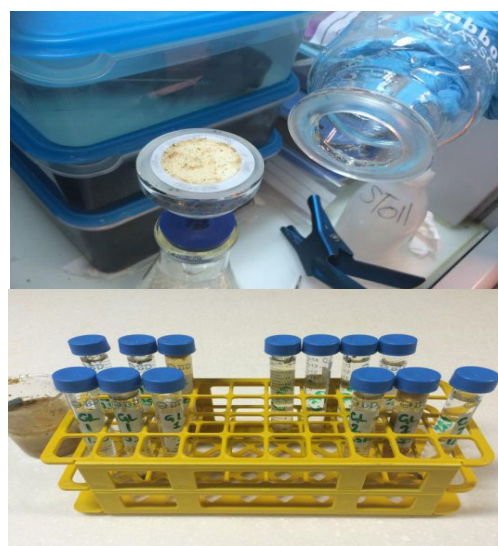
Picture 14: Filters with different size

the samples were sequentially filtered with the net size of 1.25mm, 0.5mm, and 125um as the **Picture 14** shows and generated 14 samples in total. The filtrate were preserved again at -20°C until further isolation.

k) Plastic isolation: Firstly, all the sample were put in an oven for 24 h at 60 °C. These dried materials, essentially consisting of leaves, wood pieces, planktons and plastic, was weighed after drying in order to assess the relative proportions of plastic and organic matter. Secondly, in order to remove a portion of organic matter, the dried materials were oxidized with hydrogen peroxide and sulfuric acid (35% H₂O₂, catalyzed with 20 ml of acid solution, 0.05 M Fe (We chose 98% H₂SO₄ and FeCl₃). As related research, this procedure was insufficient to oxidize polymers, but was efficient in dissolving the organic materials in the samples (Nuelle et al, 2014; Taubinger and Wilson, 1965).

Three ml of H₂SO₄ mixed with FeCl₃ were added in each sample followed by 6ml of the 30% H₂O₂ that was gently added. At least 2-3 minutes are needed to totally react between the acid and the organic material. As this reaction is very intense and dangerous, this work has to be done in a well protected space (with glasses and the special coat). Then, after the material organic digestion, the sample was carefully collected and filtered with a 0.4mm filter membrane (PC) (**Picture 15**). The material on the filter was the rest in suspension in ultra-pure water in 15 ml tube.

Picture 15: Sample filtering



Finally, samples were dried and deposited in petri dishes and selected under the microscope (**Picture 16**). For each sample dishes, the same procedure was followed. Respectively, visual observations, a microscope and magnifying glass were necessary in collecting the plastic after digestion of organic matter (The fractions



Picture 16: Plastic collection with the microscope

>5 mm were examined with the naked eye and fractions >1 mm were analyzed with microscope).

4. Results and discussion (Physico-chemical properties of nano sized latex particles)

4.1 Behavior of latex particles in ultra-pure water in pH changing conditions

a) Amidine latex particles

In this experiment, we add the Amidine Latex into the Milli-Q water. If we change the pH of this solution from 3 to 10, the Zeta potential of the latexes decreased along the pH reduction. Particularly, when we increase the pH of the solution more than 8, the zeta potential of the latex decreased correspondingly to 0 and continued to be negatively charged finally (Figure 11).

As to the latex size, however, it starts to increase along with the solution pH increase in a large scale. It means these latexes coagulated with each other when we increase the solution pH (Coagulation is due to the surface charge neutralization of the latex particles). So the hypothesis could be deduced that the originally positive charged nano plastic latex will start to coagulate in the certain situation when the pH is high enough. We can also say that the originally positive charged nano plastic latex is relatively more stable in low pH situation than in high pH condition (Figure 12).

b) Carboxyl latex particles

Contrarily, when we put the Carboxyl Latex into the MQ water, the results are quite different. On one hand, if we reduce the pH of this solution from 6 to 3, the Zeta potential of the latexes will also decrease at first along the pH reduction. However, with the pH continuously decrease, the charge of the latex also changed to positive. On the other hand, when we increased the solution pH from 6 to 11, the latex zeta potential always keep relatively stable in negative. It means that the originally negative charged plastic latex is relatively more stable in high pH situation than in low pH media (Figure 13).

Figure 11: Zeta of Amidine in Milli-Q with pH change

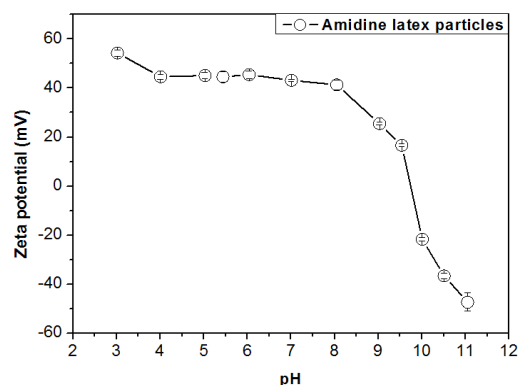


Figure 12: Size of Amidine in Milli-Q with pH change

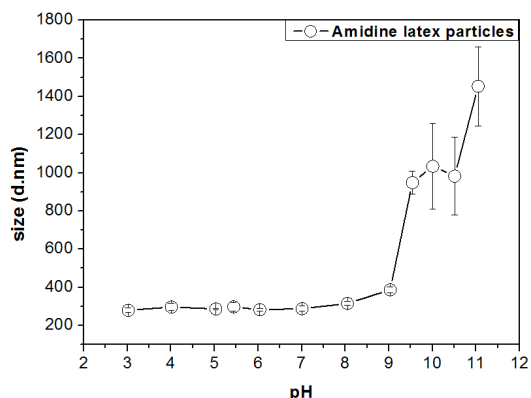
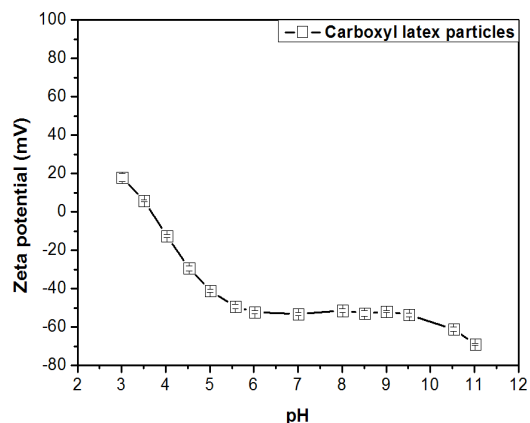


Figure 13: Zeta of Carboxyl in Milli-Q with pH change



From the Figure 14 we can see, with the pH decrease, Carboxyl latex particles start to coagulate around pH 4. So the hypothesis could be deduced that the originally negative charged plastic latex will start to coagulate when the pH is low enough. With this result, we may also say that the originally negatively charged Carboxyl latex is relatively more stable in high pH media than in low pH media.

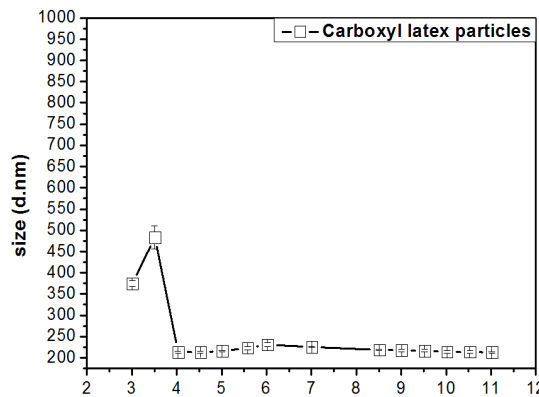


Figure 14: Size of Carboxyl in Milli-Q with pH change

For these two different latexes, they have different properties in the different pH value. The Zeta potential and the size of the Amidine and Carboxyl latex can be changed with the pH value in Milli-Q water. Coagulation happened for these two latex when we changed the media pH to an appropriate point.

4.2 Behavior of latex particles in synthetic and natural water

4.2.1 Amidine and Carboxyl in natural water and *D.magna* growth medium

a) Amidine and Carboxyl latex at 100mg/L in natural water (lake Geneva and Versoix river)

For the natural water, we used the filtered Versoix river water and Geneva lake water (with a 0.4mm sized filter pore). As these water come from different location, their properties are quite different consequently: Firstly, the concentration of the dissolved organic matter is much higher in Versoix river than the lake Geneva (CDOM in Versoix river: 2.53mg/L; in Geneva lake 0.66mg/L; in *D.magna* growth medium and MQ: 0mg/L). Secondly, the conductivity in Versoix river is also higher than Geneva lake. Thirdly, for the pH, the river water again is higher than the lake water (for the river water pH 8.34; the lake water pH 8.07). Those differences probably could influence the latex properties and that's also why we use these different milieu water to do our plastic latex experiment. We try to find related links between different water properties and the changes of the plastic latex particles in these water media.

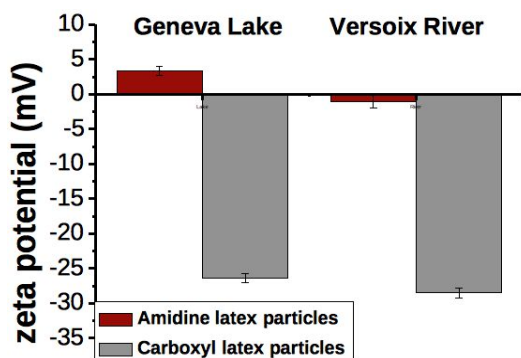


Figure 15: Zeta potential of the latex Amidine and Carboxyl in natural water

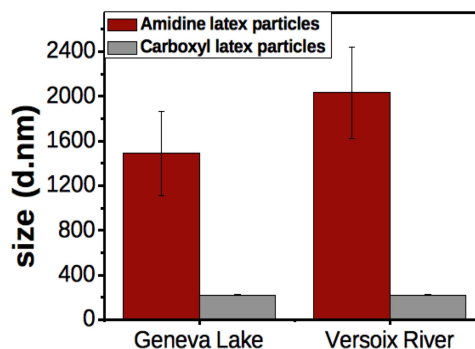


Figure 16: Size of the latex Amidine and Carboxyl in natural water

From the Figure 15 we can see that the zeta potential of Amidine latex changed with a large scale both in river water and lake. In the river water, the zeta potential of the high positively charged Amidine latex (about +40mV in Milli-Q at pH near 8) changed the charge and even to the negatively charged. In the lake water, the zeta potential changed also to the low charge near 0. For the latex size, we can see clearly from the Figure 16 that it changed in a large scale both in the river water and the lake water. However, for the latex Carboxyl, the zeta potential changed little bit both in river water and lake and not much as the changes of Amidine latex. The zeta potential of the Carboxyl latex (high negatively charged in Milli-Q water around -50 mV at pH near 8) changed with almost in same scale both in the lake water and the river water but the size keeps almost stable in the both media.

b) Comparison of Amidine and Carboxyl latex at 100mg/L in four different medium (Geneva Lake water, Versoix River water, *D.magna* growth medium, ultra-pure water)

If we compare the changes the size and zeta potential for these latexes in different mediums (natural river water, lake water and the *D.magna* grow medium), the changes can be showed by the graphic below. (By the way, it's important to note clearly the content of the *D.magna* growth medium (the OECD202 synthetic freshwater) (Table 2) before our experiments).

Ingredient (per L)	294mg CaCl ₂ *2H ₂ O, 123.25mg MgSO ₄ *7H ₂ O, 64.75mg NaHCO ₃ , 5,75mg KCl	Guideline for the Test of Chemicals/Section	2
For using	Room temperature	Effects on Biotic Systems	Daphnia sp
Preparation	Deionized water (pH : 7.8±0.2)	Acute Immobilization Test.	No. 202
Reference	OECD 2004	Conservation	+4°C in dark

Table 2: *D.magna* growth medium (the OECD202 synthetic freshwater)

(i) The zeta potential measurement of the latex Amidine and Carboxyl in different media. When we add Amidine latex into those media, from the Figure 17 we can see that the zeta potential of Amidine latex changed largely both in river water and lake water. The zeta potential in rive water even changed from positive charge to negative. However, in the *D.magna* growth medium and Milli-Q, the zeta potential did not get much change and the Zeta value keeps in high positive around +40 mV as we measured before.

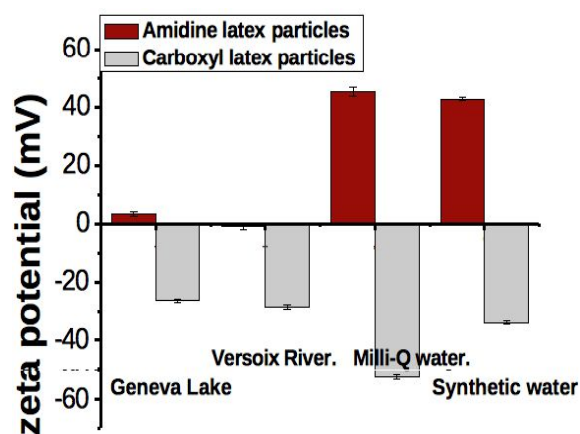


Figure 17: Zeta potential in four different solutions

However, for the Carboxyl latex, the situation of the Zeta potential is totally different with the Amidine latex. We can see from the Figure 17 that the zeta potential in lake water, river water and the *D.magna* growth medium changed in a little scale (between -25 mV to -35 mV). Comparing our earlier measurement of Carboxyl zeta potential in Milli-Q water (around -50), the zeta potential of the Carboxyl latex is relatively stable and keeps in charging negatively in those four different media. Different water properties did not change much of the Zeta potential for the Carboxyl latex.

(ii) According to the Zeta potential changes of these two latex particles in different solutions, we can see the size changes in these solutions as Figure 18 shows. Corresponding the change of the Zeta potential, the size of the Amidine latex changes largely both in the lake water and the river water. In the river water, the size changes even reached the highest level among all those changes in different solutions. Contrarily, the size in the *D.magna* growth medium and the Milli-Q water keeps stable. The properties of the latex Amidine were strongly modified in those natural river and lake water.

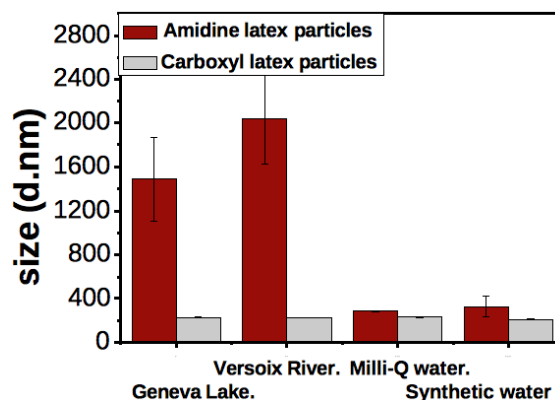


Figure 18: Size in four different solutions

For the size of latex Carboxyl, it kept very stable in different media as the Figure 18 shows. In Milli-Q water, the size of this negatively charged Carboxyl latex is about 225 (d.nm). It's property is quite different from the latex Amidine. In all those different media, the size kept very stable as origin (about 220 d.nm). So we say that the size of the negatively charged Carboxyl latex is very stable in different water milieu.

4.2.2 Behavior of the latexes during a residence (6 days) time in natural water

With the measurement results, comparing the Amidine and Carboxyl changes of size and Zeta potential, the graphic goes like the Figure 19 shows. We can see that the zeta potential of the Amidine latex decreased from high positively charge (+40 mV in Milli-Q) to negatively charged both in river water and the lake water after 6 days exposure. However, the zeta of the Carboxyl always keeps negatively charged (-50 mV in Milli-Q) both in river water and the lake water.

However, from the Figure 20 we can see that the size of the Amidine latex increased from around 220 nm to around 2000 nm. The size of the Carboxyl in lake water kept stable same as in Milli-Q (around 250 d.nm) but the size in the river water changed lightly. However, the size change of the latex Amidine was different from the Carboxyl latex and it highly increased from about 250 d.nm (in Milli-Q) to around 1400 d.nm.

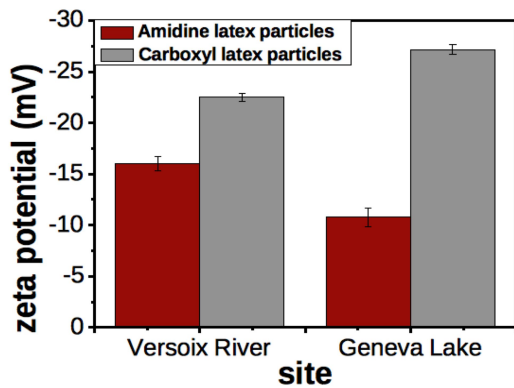


Figure 19: Zeta changes of in Versoix river and Geneva lake

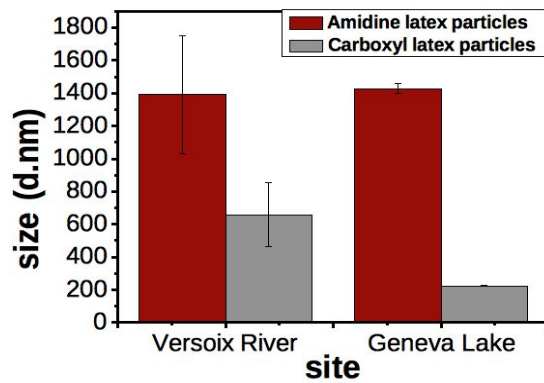


Figure 20: Size changes in Versoix river and Geneva lake

The size of the Amidine latex increased with a large scale after 6 days keeping in the natural water systems. These latexes coagulated with each other strongly in this period. During these 6 days, the biofilm around those latexes were well developed and it covered the surface of the latex and changed the characteristics of the latex from positive charge to negative charge. However, the size of the Carboxyl latex keeps stable about 220 d.nm and the zeta potential keeps constant around -27 mV. So, in this essay, we found that the property of the positively charged plastic latex are easily modified and not stable in natural aquatic systems but the negatively charged plastic latex are quite stable in natural aquatic systems.

4.2.3. Latex particles in *D.magna* growth media 12 days

For the Amidine latex, the Zeta potential decreased with the time scale from positive around 40 mV close to 0 mV during 12 days in *D.magna* growth medium. Along with the Zeta potential decrease, the latex coagulation happened and it leads to the increase in size. But the Zeta potential of the Carboxyl latex kept stable in minus (around -35 mV) as original charge. Without changes of the surface charge happened, the size of the Carboxyl in *D.magna* growth medium kept always stable (Figure 21).

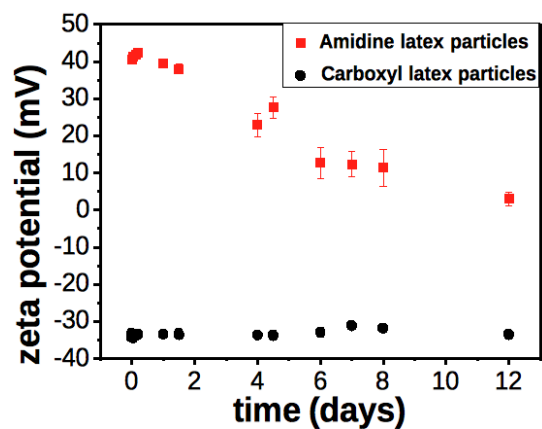


Figure 21: Zeta potential of the two latexes (20mg/L)

Correspondingly, the size of the Amidine latex in the *D.magna* growth medium increased gradually in a large scale (From 270 d.nm to 1311d.nm) during 12 days. The Amidine latex aggregated with the time scale and they aggregated into big pieces and floated surface of the solution or precipitated at the bottom. However, for the latex Carboxyl, the size kept stable around 215 d.nm during this 12 days (see Figure 22).

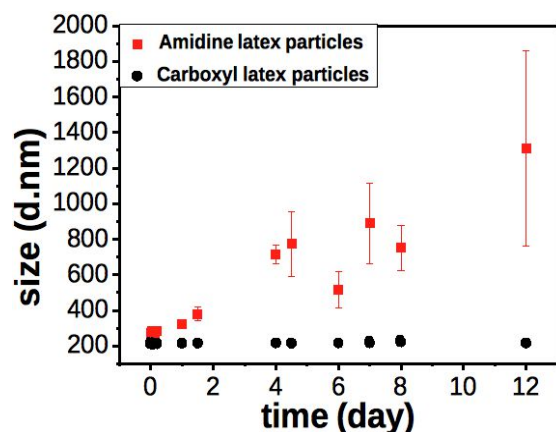
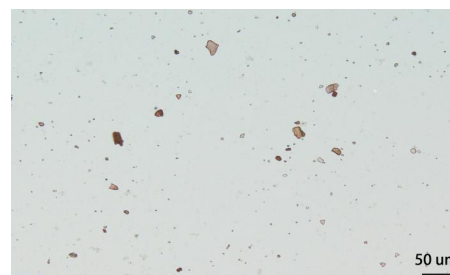


Figure 22: Size of the two latexes (20mg/L)

As we measured before, when we add the Amidine latex into the *D.magna* growth media, the zeta potential and the size did not change much. However it changed largely with the time scale of 12 days. The reason, probably, is the composition of this *D.magna* growth media (see the table 2). Once we made this fresh synthetic solution, it's pH value is about 7.2. But with the time scale in room temperature, the chemical compositions of the solution started to decompose and those decomposed anions raised the solution pH value. As the result, the Amidine latex started to aggregate in this solution when the pH reached the enough high value for them as we measured before. However, the Carboxyl latex keep stable in the basic conditions. That is why the difference happened when we measured them in a time scale.

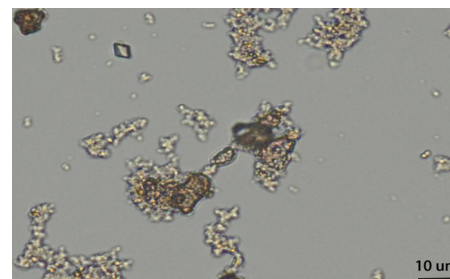
4.2.4. Latex particles aggregation behavior in various conditions

When we add the Amidine latex (100mg/L) at a concentration equal to 100mg/L in the Milli-Q water (see Picture 17), the latex are distributed and no coagulation is detected. The latex Amidine is stable in Milli-Q water (The solution pH is about 7.4).



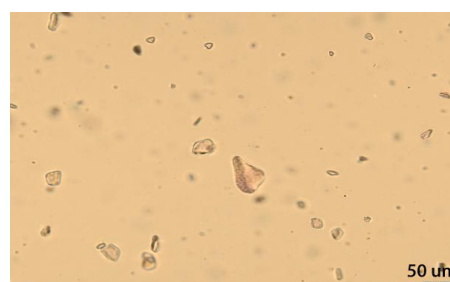
Picture 17: Amidine latex in Milli-Q

However, when we add this Amidine latex (100mg/L) in the filtered natural water (Geneva lake water), the latex concentrated in groups with the formation of aggregates (see Picture 18). The natural water modified the properties of the positively charged latex particles. (The solution pH is around 8.3)



Picture 18: Amidine latex in filtered lake water

The Figure 19 shows the Amidine latex (20mg/L) in the *D.magna* growth medium (The solution pH is about 7.4). The latex coagulated lightly and the coagulation continues with the time going on.



Picture 19: Amidine latex(20mg/L)in daphnia solution

The Picture 20 shows the Carboxyl latex (20mg/L) in the *D.magna* growth medium (The solution pH is about 7.4). There is no evident coagulation happened with the latex and the latex well distributed. It means that the Carboxyl latex is very stable in the *D.magna* growth media.



Picture 20: Carboxyl latex(20mg/L)in daphnia solution

4. 2.5. Electric charge of the Amidine and Carboxyl latex in different solution

If we put all the experiment results of those two microplastic latex in one table, we can clearly see the changes of their electrical charge in different media (Table 3).

Table 3: The links between the latex surface charge and the latex physical situation in different medium

Medium Microplastic latex	Milli Q (Deionized water)	Cultured medium (Daphne living medium)	Natural medium (river and lake)
Carboxyl (Negatively charged) —	Dispersed (Negatively charged) —	Dispersed (Negatively charged) —	Dispersed (Negatively charged) —
Amidine (Positively charged) †	Dispersed (Positively charged) †	Dispersed → Aggregated (Positively charged decreased to Negative lightly with time scale) † → — (gradually)	Aggregated seriously (Positively charged decreased to Negatively charged) † → — (quickly)

Carboxyl latex particles in Milli-Q water, Cultured *D.magna* growth media and the natural water kept stable in its' size and Zeta potential. Their surface electrical charge remained always in negative. These negatively charged latex particles were quite well distributed in those different solutions. On the contrary, the Amidine latex in Milli-Q kept positively charged as origin. However, when we put this latex in the cultured *D.magna* medium, the surface charge decreased with the time scale and started to coagulate into big pieces. When the latex was add to the natural water, the surface charge decreased quickly from positive to negative and the strong coagulation happened at the same time.

5. Ecotoxicity of latex particles with *D.magna*

5.1 The results of the experiment (*D.magna* death or immobilization)

Three parallel experiments with *D.magna magna* have been performed for each plastic latex (Amidine and Carboxyl). Figure 23 below shows the average percentage of the dead or immobilized *D.magna magna* upon exposure to increasing latex concentrations. Apparently, the *D.magna* death or immobilization rate increased along with the latex concentration augment. The *D.magna* death or immobilization percentage in Amidine latex (positively charged) is higher than that of the Carboxyl (negatively charged). The positively charged latex has stronger influence to the *D.magna* than the negatively charged latex during short term exposure (48 hours).

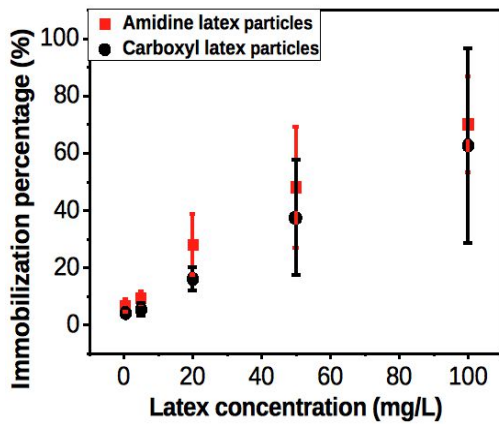


Figure 23: Ecotoxicity trend with the latex concentrations

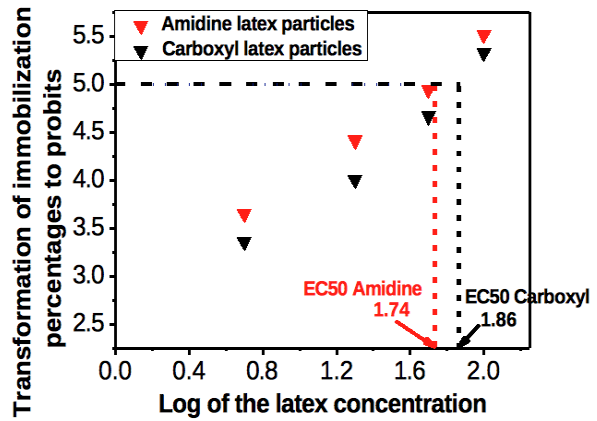


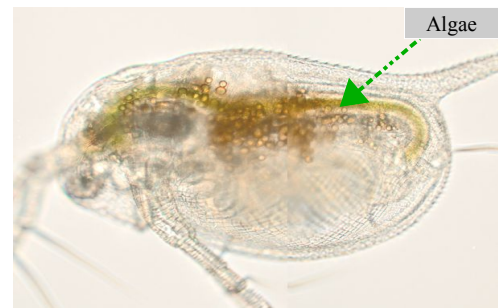
Figure 24: Ecotoxicity assay results and the EC50

We can see from the Figure 24 that the concentration can induce the toxic effect to the 50% of the organisms (EC50). EC50 of the Amidine latex is about 55mg/L (log value of 1.74 corresponding the latex concentration 55 mg/L) and for the Carboxyl latex is about 72.4mg/L (log value of 1.86 corresponding the latex concentration 72.4 mg/L) (For the protocol of the calculations, see the Annex 5). Combining this latex ecotoxicity assay results with the latex properties experiment we did before, the easily coagulated latex Amidine has stronger influence on the *D.magna* than the stable latex Carboxyl.

5.2 Uptake of Amidine latex particles by *D.magna*.

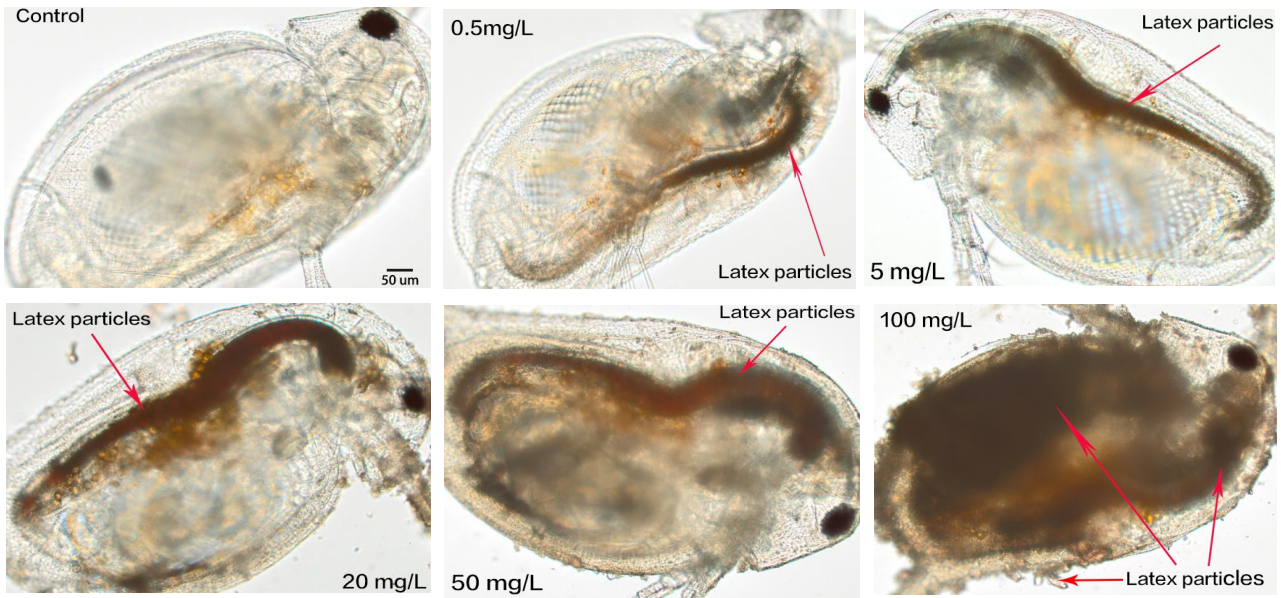
The cultures of *D.magna* were fed 2h before exposure, and their gut were filled with algae as illustrated on Picture 21. Unexposed *D.magna* controls after 48 hours, we can see that the algae has been digested partly (in the control). We observe the algae in the gut. The whole body is transparent and clean.

Picture 21: The *D.magna* was fed 2h before exposure



When the *D.magna* were exposed to 0.5mg/L Amidine latex suspensions for 48 hours, *D.magna*'s gut was filled with plastic latex (dark color in the Picture 22). Exposure to increasing concentrations of Amidine latex resulted in the complete filling of *D.magna*'s gut (exposure to 0.5mg/L, 5mg/L, 20mg/L, 50mg/L) and adsorption of latex particles at the *D.magna* surface at exposure concentration of 20mg/L. At highest exposure concentration of 100mg/L latex particles covered important surface of the *D.magna* body.

However, we should know that the latex concentrations we made for the ecotoxicity test are much higher than the microplastic concentration exists in the natural environment. A research has been done recently to measure the maximum concentration and mass of the plastic particles in the North Pacific Subtropical gyre. The results were finally recorded respectively in: 32.76 particles/m³ and 250 mg/m³ (0.25mg/L) (Goldstein et al., 2012).

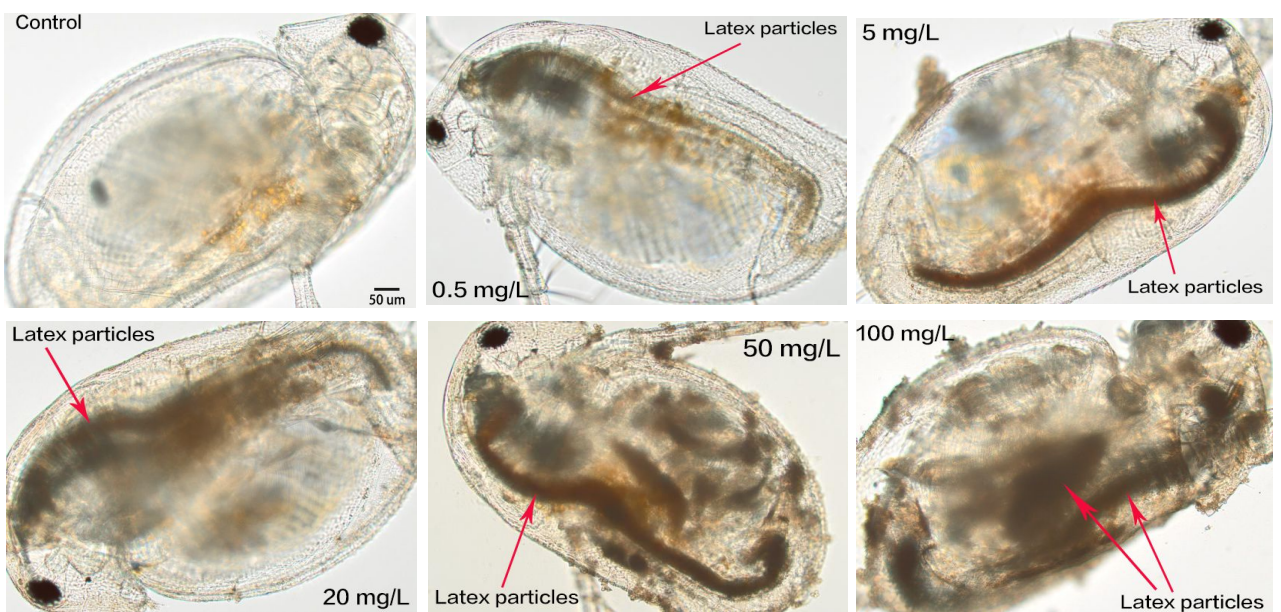


Picture 22: The photos of the *D.magna* with a 48h exposure in increasing Amidine latex concentrations

As our previous latex property experiments, the late Amidine in the *D. magna* growth media, will coagulate with the time scale. As the result, the latex size will continuously increase with the zeta potential decreasing. The hypotheses could be draw that in the high concentration latex media wells, the latexes coagulated into big pieces and probably easily filled the gut for preying by mistakes. However, in the low concentration wells, the *D.magna* can only prey those small coagulated latex parts and only partially filled their gut during the same short experiment period.

5.3 Uptake of Carboxyl latex particles by *D.magna*.

Same essay was also done with the Carboxyl latex. From the *D.magna* body in the control, we can see that the algae has been almost totally digested and the whole body is transparent and clean.



Picture 23: The photos of the *D.magnas* with a 48h exposure in increasing Carboxyl latex concentrations

Starting with the exposure of 0.5mg/L latex concentration for 48 hours, *D.magna's* gut was filled with plastic latex (dark color from Picture 23) as the essay in Carboxyl latex media. With the latex concentrations increasing from 0.5mg/L to 100mg/L, the quantity of the consumed latex was augmenting correspondingly and the latexes were adsorbed seriously at the *D.magna's* surface as well. At the highest exposure concentration of 100mg/L, latex particles strongly covered the surface of the *D.magna* body and the immobilization of *D.magna* reached the highest level .

As the latex property experiment, we know that the Carboxyl latex was quite stable in the *D.magna* growth media, even with the time scale. The size and the zeta of the latex always keep in the same level. The latex particles are well distributed. However, comparing the experiment with the Amidine latex, the latexes coagulated with time scale in the *D.magna* growth media. This is, probably, one of the reasons why the *D.magna* in the Amidine latex solution were more strongly influenced and there was higher immobilization rate. The *D.magna* consumed big coagulated latex pieces and has much higher latex concentration than that in the Carboxyl latex media. It is also maybe the main reason why the EC50 in Carboxyl latex media is higher than the EC50 in Amidine latex solution during a short term exposure.

6. Plastic collection and isolation in Geneva Lake

In marine water, the micro-plastic pollution has attracted more and more public and scientific interests in the last few years. The situation, however, in freshwater environments remains largely unknown although the plastic pollution in freshwater plays an important role as a part of the origin of marine pollution. As the importance of this pollution in the freshwater systems, a field research has been taken to identify the potential impacts and specify the situation for Lake Geneva. The surface water of Geneva Lake has been sampled using a floating manta net. Finally, the collected plastics have been sorted in types (fragments, pellets, cosmetic beads, lines, fibers, films, foams). From our sampling results, it seems that all the sampling sites of the Geneva Lake are affected by this pollution and different plastic types have been found in all samples.

6.1 Result of the plastic quantity and types

Under the microscope, plastics were identified from organic materials and classified into different categories according to their appearance, types and possible origin. The most commonly considered plastics were found and detailed into 7 categories (see photos blow). In total, we collected 0.093g (more than 1.2%) plastic from the total mass of 7.53g dried mixed material from the 4 sampling sites. 196 pieces (69 macro-plastics, 127 large micro-plastics) of different particles we observed finally (Photos below).

a) Different fragments from the Picture 24 are mainly degraded from large plastic particles. They have a diverse source and probably come from the land dumping, run off, or fishing. The size of these plastic is from 0.5mm to 3.5mm and the colors was in a large diversity.



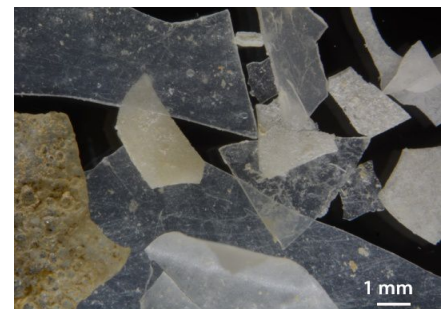
Picture 24

b) Plastic foams (Picture 25) mostly come from the construction areas, such as the isolation and protection products. Their size is sorted from 0.6mm to 4.8mm. Foam (expanded polystyrene), widely used in the field of construction, building (insulation and sheeting, respectively) and the food industry. These plastics are usually stored and worked in the open air, paving the way for their dispersion by air or by water.



Picture 25

c) The thin transparent film (size from 0.4mm to 9.8mm) which is composed mainly of PE mostly comes from the packaging (Picture 26). For example, a large number of this pollutants comes from the food production market.



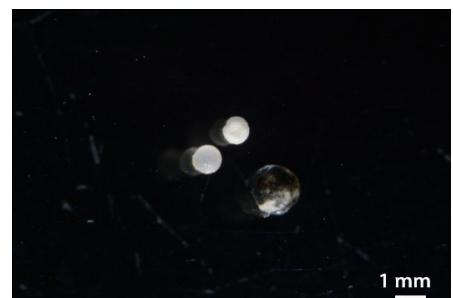
Picture 26

d) The plastic granules (Picture 27) are sorted with size from 0.3mm to 3.1mm. They can be in different colors. This kind of the plastic mainly comes from different land industry or related constructions.



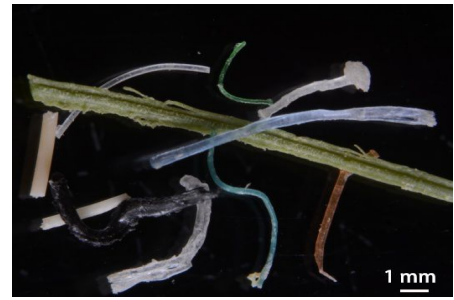
Picture 27

e) The pellets (Picture 28) are sorted from the size 0.6mm to 1.1mm. These plastic pellets come from industry but mostly from the pre-productions or raw materials. The cosmetic products contribute to a large amounts of this pollution nowadays.



Picture 28

f) The fishing lines (Picture 29) are sorted with size from 0.3mm to 26mm. These plastic fishing lines mostly come from the product packaging or fishing industry.



Picture 29

g) The textile fibre (Picture 30) is sorted with size from 0.2mm to 7.8mm. This type of the plastic comes from the multi-origin. For example, laundry contributes to this kind of pollution in a large scale. It is the biggest part (around 60% in number) among all our collected different plastics.



Picture 30

Plastics can be from industry, construction, raw material, fishing cosmetics, textile fiber, etc. For each type, size and the number of particles was counted and analyzed. All these plastic particles are analyzed and sorted in the end, with the help of microscope photonics and the chemical laboratory.

7. Conclusion

For the different latexes, their behaviors are highly depend on the surface functional group (surface charge) and the properties of the milieu (e.g. pH, Dissolved Organic Matter, etc) where they were measured. For the positively charged Amidine, the fate was quickly modified in the natural water. It can be stable in the acid solution but quite unstable in the basic solution. It's fate can be also varied in the *D.magna* growth media with a time scale as this synthetic solution would change to light basic with time scale. For the negatively charged Carboxyl latex, it exists stable in the basic solution but unstable in the acid solution. During our tests, this latex kept stable Milli-Q water, Synthetic water and natural water as well. The dissolved organic mater in their medium is probably also one of the factors in influencing the fate of the latexes.

Our short-term ecotoxicity experiments with *D.magna* revealed that the EC50 for both Amidine and Carboxyl latex micro-particles were below 100mg/L, ranking them as harmful to organisms. However, it is difficult to say that Amidine latex is more harmful than the Carboxyl latex just with the trend of the immobilization percentage in the Amidine latex solution as we got a much larger scale of the Standard Deviation value in the Amidine latex than the Carboxyl latex. But as the behaviors of these two different latexes in different mediums, the Amidine latex will coagulate in the natural water systems quickly and will also coagulate slowly in the *D.magna* growth medium and precipitate at the bottom of the water column. As this coagulation phenomenon, the Amidine

latexes may be less available to the micro-organisms than the Carboxyl latex if we take a long term view.

From our filtering work on the Geneva, Lake we found quite diverse categories of plastic. Considering the location of Geneva Lake, it's circulated by human habitats all around. As this reason, those plastics may originate from either voluntary or involuntary urban discharges and surface runoff, but also enter waterways through drainage systems, sewage treatment, etc. Once those pollutants introduced into rivers and lakes, plastic debris may sink, be deposited on river banks and/or undergo transfer to the marine environment finally.

With our plastic collection results, apparently, the plastic concentration in the Geneva Lake is far much lower than the concentrations we made in the latex ecotoxicity test with *D.magna*. However, with the time scale, more and more microplastics will reach and accumulate in the Geneva Lake. In the sea water, the maximum concentration and mass of the plastic particles in the North Pacific Subtropical gyre respectively in 32.76 particles/m³ and 250 mg/m³ (0.25mg/L) (Goldstein et al., 2012). Although the microplastic concentrations in the natural aquatic systems are much lower than the concentration we made for ecotoxicity test, more and more micro and macro plastics will enter and accumulate in the natural aquatic systems and influence the aquatic organisms finally.

8.Perspective

Plastics are used by almost all end-use segment of the economy and their usage is quite likely to increase with the developments in the plastic industry, which in turn causes increase of plastic wastes. Plastics are associated with at least 78% of the priority pollutants and 61% of priority substances listed as toxic by the USEPA (United States Environmental Protection Agency) and EU (Olympic, 2013). Since they are found to alter the working of ecosystem, prevention/minimization of plastic becoming waste and recovery of this ecologically hazardous waste should be taken into account instead of being left freely in nature or landfilled. The public health risks resulted from PS pollution and its adverse environmental impacts should be considered, with more stringent usage of such products by today's society. Available waste management systems include recycling and/or energy recovery operations, but they need to be improved more. Obviously, new policies on plastic producing, using, recycling and plastic polluted water treatment should thus be also developed before plastic waste becomes an unsolvable problem (Gürü et al., 2014).

Importantly, the floating plastic marine debris will never completely disappear from the environment because of its durability, although macro-plastic debris may gradually be changed to meso-, micro-, or nano-plastic as a result of various degradation processes (Barnes et al. 2009). As

long as humans continue to produce plastics, the volume of plastic marine debris will continue to accumulate in the environment (Kako et al., 2014).

Efforts to combat plastic pollution are already underway at different levels of the local communities. These are mainly focused on (1) regular removing garbage from the land and water near urban centers, (2) improving the recycling process, and (3) education. Initial results of the social networking effort are beginning to take shape through school involvement, citizens sharing knowledge with each other, and progress made in concerted, collaborative actions taken by the communities. Improvement of the coordination of social agents, governmental administrations, civil society, and other stakeholders in order to change the trajectory of ever-increasing plastic waste, and commit to reducing plastic pollution to zero. Such collaborative approaches to this problem are necessary to determine possible solutions and convince state agencies to assume their responsibilities with regards to citizens' health, and the environmental impact (Baztan et al., 2014). Unfortunately, these efforts are not enough. Furthermore, Global Environmental concern also always hit a wall as no global legislation exist. There is no real consequences "legally driver" exist if no action is taken by us.

According to our research, there are four advisable solutions:

- 1) Improve the implementation of protocols, directives, and relative laws. For example, one of the remarkable milestones has to be noted here that the U.S just banned the plastic microbeads in the face wash (Zoé. S., 2015). President Barack Obama signed a bill into law in December 2015 banning them for good. The new bill, called the Microbead-Free Waters Act of 2015, requires manufacturers to eliminate microbeads from their products by 2017.
- 2) Reduce industrial and domestic use of plastic. At same time, use of effective product such as bioplastic which is derived from renewable biomass sources, such as vegetable fats and oils, corn starch, or microbiota. For example, there are more and more encouraging news that the increasing enterprises stop serving plastic bags for purchasers. People are encouraged to replace plastic products with bio-alternatives.
- 3) Increase awareness of plastic pollution hazards for all levels of society and for all stakeholders. The knowledge of plastic pollution hazards should be popularized by different government departments, academic institutes, non governmental associations, organizations, etc. Furthermore, all of them should be well encouraged by government as well from economic supporting, necessary facilities conveniences, etc.
- 4) Develop collective, collaborative, and concerted actions between stakeholders.

Considering the plastic pollution needing a global cooperation, according to Rochman (2013): "the physical dangers of plastic debris are well enough established, and the suggestions of the

chemical dangers sufficiently worrying, that the biggest producers of plastic waste — the United States, Europe and China — must act now. These countries should agree to classify as hazardous the most harmful plastics, including those that cannot be reused or recycled because they lack durability or contain mixtures of materials that cannot be separated” (Rochman et al., 2013).

Since 1990, the dumping of rubbish at sea from ships has been prohibited under the international shipping regulation MARPOL ANNEX V. A reduction of ship-derived plastic debris should therefore be expected, even if global use of plastics continues to increase. To gain an accurate and meaningful assessment of plastics and their influence, large-scale and long-term monitoring is needed across countries and continents (Barnes et al., 2009). Furthermore, associated effects by transport of other contaminants (organics, metal) and the creation of a new ecological niche is clearly understudied. The battle against plastic has clearly started and more effective efforts are required to control plastic pollution and its impact on our aquatic environment.

9. Annexes

1) Amidine Latex property details

Catalog Number	A37314
Product Name	Amidine latex, 4% w/v 0.2 µm
Molecular Formula	N/A
Appearance	suspension
Medium	de-ionized water
Lot Number	1627736

Positively charged polystyrene microspheres with amidine functional groups on the surface.
 Surface charge is pH dependent. Stable at low pH. Avoid using multivalent anions. Avoid using glass vessels. Surface is hydrophobic in nature.
 STORE AT 2 - 8°C, DO NOT FREEZE

	LOT DATA	SPECIFICATION
PHYSICAL PROPERTIES OF PS¹		
Density at 20°C	1.055 g / cm ³	n.a.
Refractive Index at 590 nm, 20°C	1.591	n.a.
TECHNICAL DATA		
Material Lot Number	2503	n.a.
Mean Diameter (TEM) ²	0.22 µm	0.15 - 0.25 µm
Standard Deviation of Diameter	0.010 µm	n.a.
Coefficient of Variation of Diameter	4.3 %	≤15 %
Percent Solids w/v	4.3 %	4.0 ± 0.5 %
Amidine Charge Titration Data	35.3 µEq / g	n.a.
Bioburden Test	meets specification	0 CFU / mL
THE CALCULATED DATA		
Particle Number per Milliliter of Latex	7.3 x 10 ¹²	n.a.
Specific Surface Area	2.6 x 10 ⁵ cm ² /g	n.a.
Surface Charge Density	13.2 µC/cm ²	n.a.
Parking Area per Amidine Group	122 Å ² / C(NH)NH ₂	n.a.
Charge Groups per Particle	1.2 x 10 ⁵	n.a.

2) Carboxyl Latex property details

Catalog Number	C37486
Product Name	Carboxyl latex, 4% w/v 0.2 µm
Appearance	white suspension
Medium	de-ionized water
Lot Number	1704377

Negatively charged polystyrene microspheres with carboxyl functional groups on the surface.
 Surface charge is pH dependent. Stable at neutral to high pH. Surface is hydrophobic in nature.
 STORE AT 2 - 8°C, DO NOT FREEZE

	LOT DATA	SPECIFICATION
PHYSICAL PROPERTIES OF PS¹		
Density at 20°C	1.055 g / cm ³	n.a.
Refractive Index at 590 nm, 20°C	1.591	n.a.
TECHNICAL DATA		
Material Lot Number	2549,1	n.a.
Mean Diameter (TEM) ²	0.21 µm	0.15 - 0.25 µm
Standard Deviation of Diameter	0.011 µm	n.a.
Coefficient of Variation of Diameter	5.0 %	≤12 %
Percent Solids w/v	4.1 %	3.5 - 4.5 %
Carboxyl Charge Titration Data	28.8 µEq / g	n.a.
Bioburden Test	meets specification	0 CFU / mL
THE CALCULATED DATA		
Particle Number per Milliliter of Latex	8.0 x 10 ¹²	n.a.
Specific Surface Area	2.7 x 10 ⁵ cm ² /g	n.a.
Surface Charge Density	10.3 µC/cm ²	n.a.
Parking Area per Carboxyl Group	156 Å ² / COOH	n.a.
Carboxyl Groups per Particles	8.9 x 10 ⁴	n.a.

3) CML Latex property details

Catalog Number	C37480
Product Name	CML latex, 4% w/v 0.2 µm
Appearance	white suspension
Medium	de-ionized water
Lot Number	1405624

Negatively charged polystyrene microspheres with sulfate and high density of carboxyl functional groups on the surface.

Surface charge is pH dependent. Stable at wide range of pH. Surface is hydrophobic at low pH and somewhat hydrophilic at high pH.

STORE AT 2 - 8°C, DO NOT FREEZE

	LOT DATA	SPECIFICATION
PHYSICAL PROPERTIES OF PS¹		
Density at 20°C	1.055 g / cm ³	n.a.
Refractive Index at 590 nm, 20°C	1.591	n.a.
TECHNICAL DATA		
Material Lot Number	1180577	n.a.
Mean Diameter (TEM) ²	0.20 µm	0.15 - 0.25 µm
Standard Deviation of Diameter	0.009 µm	n.a.
Coefficient of Variation of Diameter	4.6 %	≤12 %
Percent Solids w/v	4.0 %	3.5 - 4.5 %
Carboxyl Charge Titration Data	930.1 µEq / g	n.a.
Bioburden Test	meets specification	0 CFU / mL
THE CALCULATED DATA		
Particle Number per Milliliter of Latex	9.0×10^{12}	n.a.
Specific Surface Area	$2.8 \times 10^5 \text{ cm}^2/\text{g}$	n.a.
Parking Area per Carboxyl Group ³	$5 \text{ \AA}^2 / \text{COOH}$	n.a.
Carboxyl Groups per Particles	2.5×10^6	n.a.

4) General analysis results of microplastic study in the Switzerland lakes

(1) Plastic on the surface of the lakes of Switzerland

Lac	n	Unité	Microplastiques			Macroplastiques		
			Moyenne	Médiane	Ecart-type	Moyenne	Médiane	Ecart-type
Lacs suisses	27	[#/km ²]	91'000	48'000	120'000	1'800	860	3'100
		[mg/km ²]	26'000	8'500	33'000	44'000	12'000	80'000
Léman	4	[#/km ²]	220'000	220'000	160'000	2'300	2'400	1'700
		[mg/km ²]	46'000	57'000	25'000	44'000	50'000	33'000
Léman (Petit lac)	4	[#/km ²]	33'000	14'000	46'000	1'100	835	710
		[mg/km ²]	10'000	9'400	11'000	27'000	25'000	23'000
Constance	3	[#/km ²]	61'000	63'000	12'000	830	390	1'100
		[mg/km ²]	45'000	54'000	31'000	16'000	3'200	25'000
Neuchâtel	3	[#/km ²]	61'000	62'000	24'000	290	0	500
		[mg/km ²]	7'600	7'000	3'000	1'900	0	3'300
Majeur	4	[#/km ²]	220'000	220'000	150'000	6'500	5'300	6'300
		[mg/km ²]	69'000	77'000	48'000	170'000	140'000	160'000
Zurich	5	[#/km ²]	11'000	9'800	2'600	580	0	980
		[mg/km ²]	3'700	2'800	4'200	13'000	0	28'000
Brienz	4	[#/km ²]	36'000	30'000	23'000	950	1'200	630
		[mg/km ²]	4'500	4'100	3'4 00	28'000	26'000	27'000
Erié	8	[#/km ²]	110'000	-	170'000	-	-	-
Hövs göl	8	[#/km ²]	20'000	-	-	-	-	-

(2) Plastic on the beach of the Switzerland lakes

Lac	n	Valeur	Microplastiques		Macroplastiques	
			[#/m ²]	[mg/m ²]	[#/m ²]	[mg/m ²]
Lacs suisses	33	Moyenne ± écart-type	1'300 ± 2'000	920 ± 1'500	90 ± 250	14'000 ± 33'000
		Médiane	270	110	11	480
		Min-Max	20 - 7'200	1 - 6'000	0 - 1'300	0 - 150'000
Léman	6	Moyenne ± écart-type	2'100 ± 2'000	960 ± 1'100	35 ± 65	18'000 ± 40'000
		Médiane	1'700	6'000		
		Min-Max	78 - 5'000	19 - 2'900	0 - 170	0 - 100'000
		Min-Max	140 - 620	23 - 750	0 - 22	0 - 4'800
Neuchâtel	4	Moyenne ± écart-type	700 ± 1'100	920 ± 1'700	17 ± 26	6'400 ± 13'000
		Médiane	220	110	6	49
		Min-Max	67 - 2'300	1 - 3'500	0 - 56	0 - 25'000
Majeur	9	Moyenne ± écart-type	1'100 ± 2'300	450 ± 870	28 ± 42	26'000 ± 54'000
		Médiane	180	76	11	780
		Min-Max	20 - 6'900	3 - 2'600	0 - 120	0 - 150'000
Zurich	4	Moyenne ± écart-type	460 ± 350	380 ± 670	3 ± 6	690 ± 1'400
		Médiane	480	69	0	0
		Min-Max	89 - 800	16 - 1'400	0 - 11	0 - 2'800
Brienz	6	Moyenne ± écart-type	2'500 ± 3'000	2'400 ± 2'700	400 ± 510	16'000 ± 12'000
		Médiane	1'200	1'800	190	18'000
		Min-Max	89 - 7'200	14 - 6'000	11 - 1'300	480 - 28'000
Garde (IT) 1	3	Moyenne ± écart-type	110 ± 55		8.3	
Garde (IT) 2	3	Moyenne ± écart-type	1'100 ± 980		480 ± 240	
Océan			0.21 - 77'000			

5) Transformation probit for the determination of EC50

Annexe 8 : Transformation probit pour la détermination de l'EC50

- **Etape 1 : Convertir le % de mortalité (ou d'inhibition) en probits**

Déterminer les probits en recherchant la valeur correspond au pourcentage de réponse dans la table de Finney (Finney 1952)

Table 3.2 Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.30	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

- **Etape 2 : Calculer le log des concentrations utilisées**

Le log de chaque concentration peut être calculé grâce à la formule « LOG » d'excel.

*** Le groupe travaillant sur les mélanges choisira soit le Cu soit le DDC pour représenter ses résultats***

- **Etape 3 : Tracer la courbe des probits en fonction du log des concentrations et ajouter une courbe de tendance linéaire.**

Sur Excel, tracer la courbe des probits déterminés grâce au tableau précédent en fonction des logs des concentrations calculés.

Sur le graphique, ajouter la courbe de tendance linéaire et faire apparaître l'équation de la courbe et le coefficient de détermination sur le graphique.

- **Etape 4 : Calcul de l'EC50**

A partir de l'équation de la courbe = $ax + b$, calculer la valeur de x pour laquelle le probit $y = 5$. Cette valeur correspond au probit pour lequel le log de la concentration x est égal au log de l'EC50.

Prendre ensuite l'inverse du log pour cette valeur x .

Et voilà ! Vous obtenez l'EC50 !

10. References

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