

## An ecotoxicological risk model for the microplastics in arctic waters

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### ABSTRACT

The risk posed to Arctic marine life by microplastics, a Contaminants of Emerging Arctic Concern (CEAC), is poorly known. The reason is the limited understanding of the dose-response relationship due to the region's peculiar environmental and geophysical properties and the unique physiological properties of the species living there. The properties of microplastics in the region and their distribution across the oceanic profile further complicate the problem. This paper addresses the knowledge gap by proposing a novel comprehensive ecotoxicity model. The model uses oxidative stress caused by the Reactive Oxygen Species (ROS) to assess cell mortality. Cell mortality has been used as an indicator of ecological risk. The model is implemented in the Bayesian Network (BN) framework to evaluate the cytotoxicity, measured as the probability of causing mortality. The work enhances the understanding and assessment of the cytotoxicity of microplastics in polar cod and associated risks.

### 1. Introduction

Plastics are a wide class of organic compounds. They are polymers of fossil feedstock like natural gas, coal, and oil that can be moulded into any shape when soft and then set into a rigid shape. The commonly used plastics are - polypropylene, polystyrene, low-density polyethylene (LDPE), high-density polyethylene (HDPE), and Polyethylene terephthalate (PET). Plastics are used widely for various reasons, primarily convenience (Hahladakis et al., 2018). At present, more than 5000 different types of plastics are available in the market. Industrial production of plastic has skyrocketed since the mid-20th century, reaching an annual production of 368 million tonnes by 2019 (Plastics Europe, 2020). COVID-19 witnessed a meteoric rise in plastic consumption in various forms like face masks, testing materials, and personal protective equipment. An increase in the consumption of take-away food from restaurants packed with plastic utensils and an uptick in online purchases resulted in greater plastic waste generation (Ammendolia et al., 2021).

Plastic debris or microplastics are found everywhere, in oceans,

freshwater systems, and soil globally (Andrady, 2017; Dris et al., 2016; Ivar Do Sul & Costa, 2014; Liu et al., 2018; Talbot & Chang, 2022; Xu et al., 2022). It is also an integral part of municipal waste everywhere. Around 19–23 million metric tonnes of plastic waste flow annually from terrestrial sources to oceans (Borrelle et al., 2020). Apart from land sources, maritime operations like fishing, aquaculture, shipping, and offshore fishing and oil and gas extraction also contribute significantly to ocean plastic accumulation.

Plastic is generally very durable, and thus, tends to persist for a very long time in the environment. Also, its ability of long-range transportation clubbed with the property of bioaccumulation makes it even more detrimental to animals, human beings, the environment, and the whole ecosystem. Plastics being released into the environment are exposed to several natural processes like decomposition by microbes, mechanical forces (wind, water currents, animals), chemical weathering, UV exposure, temperature variations, and photo-oxidation. This leads to the disintegration and fragmentation of plastic fibers into smaller pieces (Hidalgo-Ruz et al., 2012; Kubowicz & Booth, 2017). When the fibers reach the size of less than 5 mm, they are called

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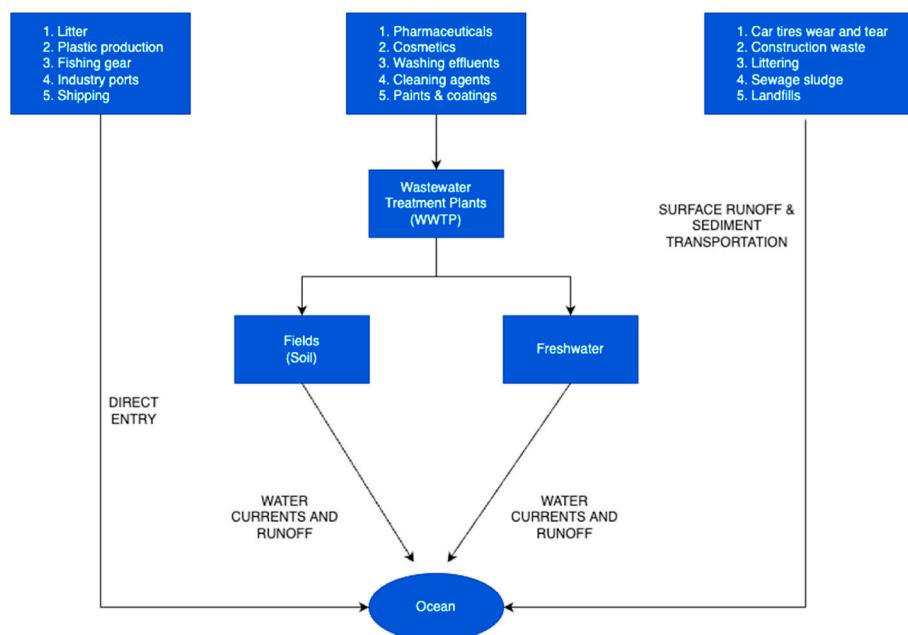


Fig. 1. Sources of microplastics in Arctic Region.

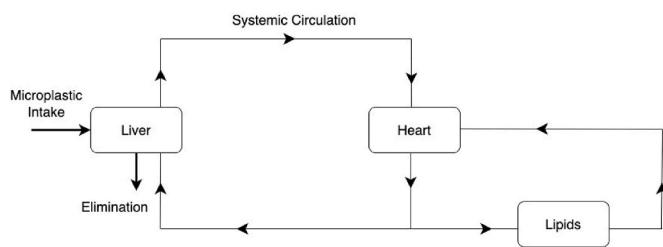


Fig. 2. Route of microplastic in polar cod after oral ingestion.

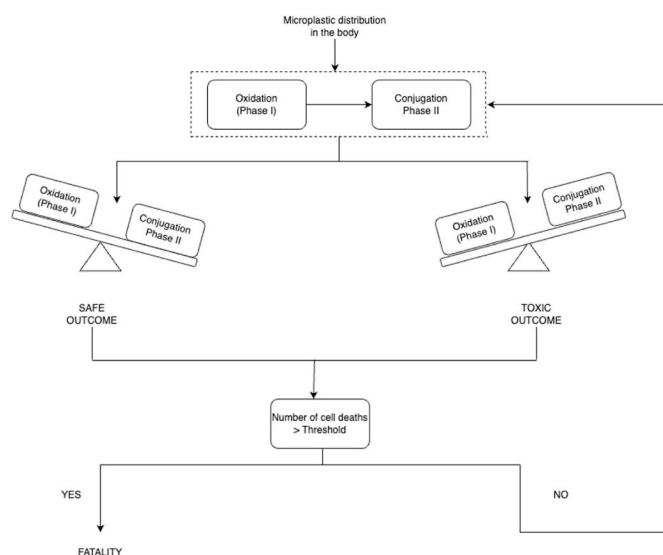


Fig. 3. Toxicity mechanism in polar cod.

microplastics (MPs). Plastic fibers in the range of 1  $\mu\text{m}$ –5 mm are referred to as microplastics (Frias & Nash, 2019). MPs are very heterogeneous when it comes to their physical and chemical properties. MPs travel through various media into the Arctic Ocean. The cold

environmental conditions of the Arctic are conducive to its accumulation (Peeken et al., 2018). reports that certain regions in the Arctic, like Fram Strait, have some of the world's highest microplastic deposition. Here, it gets introduced to the Arctic food chain. Arctic communities at the topmost trophic level of the food chain are eventually exposed to these microplastics.

Microplastics have been found in Arctic surface water (Kanhai et al., 2018), sub-surface waters (Morgana et al., 2018), the seafloor (Bergmann et al., 2017), and even ice (Obbard et al., 2014). (Huntington et al., 2020) has reported that the amount of microplastics or other anthropogenic particles is 90% for surface water and zooplankton samples and 85% for sediment samples (Cole et al., 2011). categorizes these sources into primary and secondary sources. Primary sources are those where the plastics were originally produced as microplastics, while secondary sources are those where the large plastic fibres degrade into smaller fragments (<5 mm), thereby converting them to microplastics. Ocean currents eventually fragment the larger plastic debris dumped in oceans into microplastics. To understand the gravity of this degradation (Koelmans et al., 2017), state that 99.8% of the plastic that entered the oceans from 1950 has already degraded into microplastics or even nano plastics.

Broadly, three types of compounds can be associated with marine plastic litter. First is the plastic polymer itself. Second are chemicals like plasticizers, antioxidants, and flame retardants that are added intentionally during their production for example, polybrominated diphenyl ethers (PBDEs), chlorinated paraffin, polychlorinated biphenyls (PCBs) and polychlorinated naphthalene (PCNs). The third class is hydrophobic chemicals sorbed by the microplastics from the surroundings. These include endocrine disrupting chemicals (EDCs) like polychlorinated biphenyls (PCBs), hydrophobic organic compounds (HOC) like polycyclic aromatic hydrocarbons (PAHs), heavy metals like Pb, Fe and persistent organic pollutants (POPs). All three chemicals together make microplastics very toxic to the exposed organisms (Li et al., 2018).

It is critical to assess the ill impacts of this microplastic accumulation, particularly in the pristine and sensitive Arctic region. MPs are ingested or inhaled by marine species, and thus they get into the food chain and food web of the region (Avio et al., 2020). demonstrated the presence of microplastics in various benthic, demersal, and benthopelagic or pelagic organisms. Studies like (Jiang et al., 2020) and (Gertenbacher et al., 2022) studied and reviewed the toxicological effects of

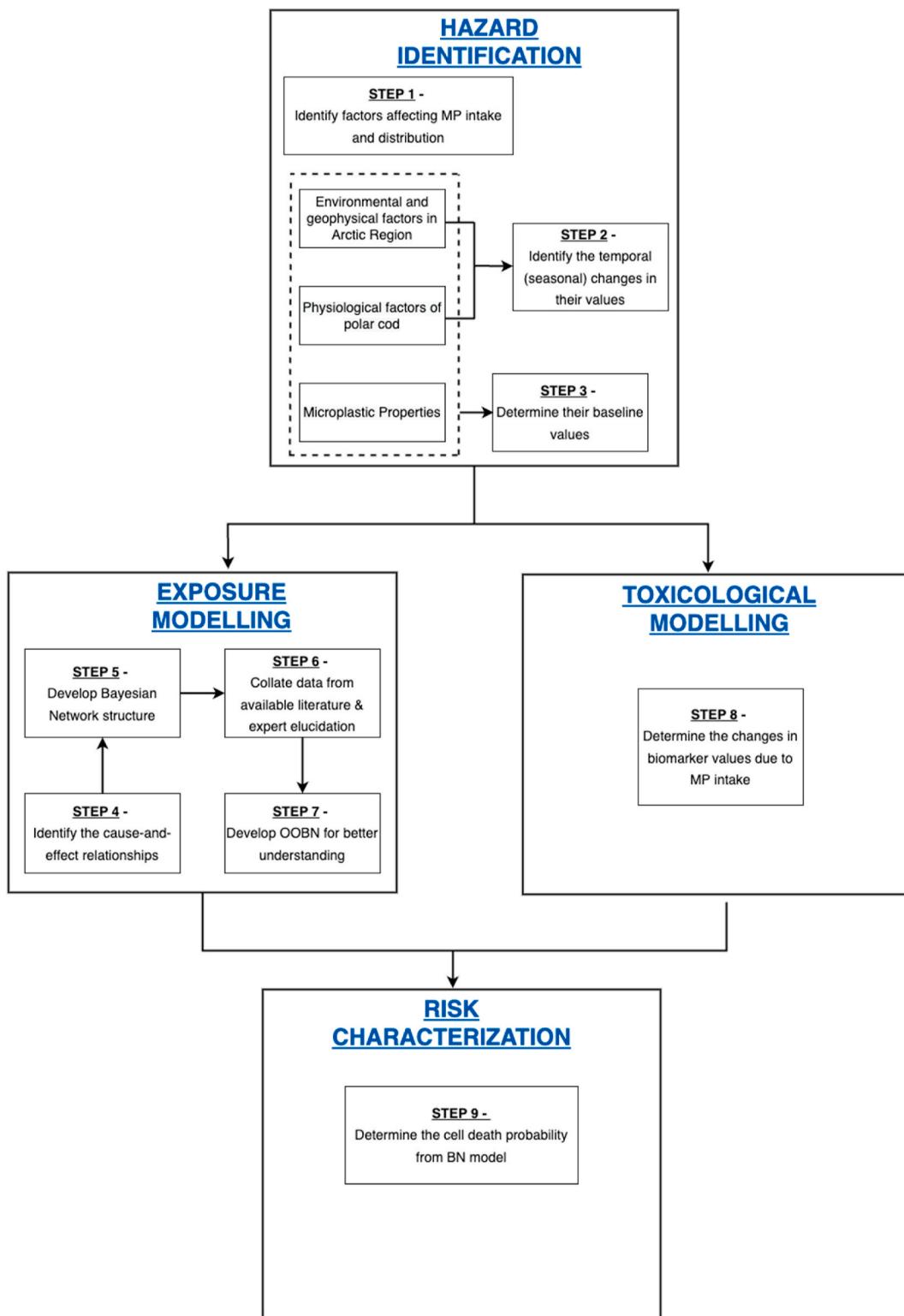


Fig. 4. Methodology of the developed ecotoxicity model.

microplastics on organisms.

Microplastics contain certain harmful chemicals like additives, flame retardants and colourants. Also, they have a tendency to attract other pollutants and harmful micro-organisms from the surroundings onto themselves. Thus, they become even more dangerous on being ingested by living beings.

### 1.1. Microplastics in the Arctic Region

The Arctic region is the area surrounding the north pole. There is no consensus on the southern boundary of the region. However, mostly it is understood as the region above the arctic circle, which is at  $66^{\circ} 33'49''$  N. As the Arctic is sparsely populated, low plastic accumulation in the region would be expected. However, extensive microplastic deposition

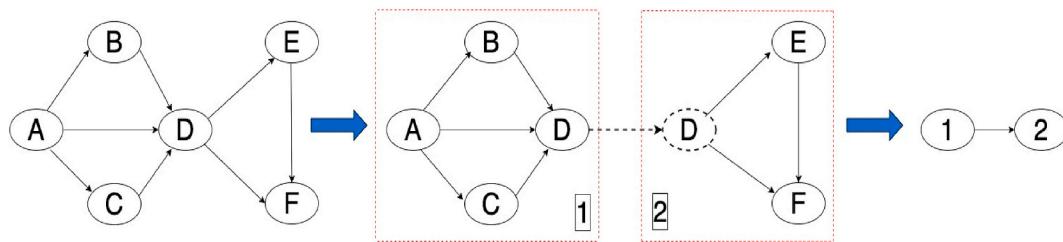


Fig. 5. Conversion of a BN to OOBN.

is observed in the region. In fact, Fram Strait in the Arctic has reported very high microplastic concentration, at  $1.2 \times 10^{-7} m^3$  (Peeken et al., 2018). Most of the microplastic in the Arctic originates from remote sources. The Arctic receives more than 10% of the global river discharges even though it contains a little over 1% of the global ocean water (Holmes et al., 2012). This makes it more prone to plastic input. Recently, due to human intervention in the region, local sources of microplastics have also contributed to it. Maritime activities like aquaculture, hydrocarbon exploration, and ship traffic contribute as local microplastic sources. Fishing gear is a primary local microplastic source in regions like Greenland, Norwegian and Barents Seas (Linnebjerg et al., 1991), Kara Sea (Benzik et al., 2021) and subarctic North Atlantic (Buhl-Mortensen & Buhl-Mortensen, 2017) and North Pacific oceans (Polasek et al., 2017). (Rist et al., 2020) identified Nuuk, the capital of Greenland, as a major local source. Fisheries are a significant source on the beaches of Svalbard, Novaya Zemlya, Franz Josef Land, and Barents Sea. Domestic sources from Arctic communities also contribute to microplastic accumulation. Plastic particles exuviate from ship paint, and skidoos are also a potential microplastic source. Greywater released from vehicles operating in the region also leads to microplastic deposition (UNEP, 2016). Southwest Greenland and Tasiujarjuaq, Nunavut, have reported paint-derived microplastic fragments (Liboiron et al., 2021). Fig. 1 summarizes all the sources of microplastic in the Arctic region.

It is vital to assess the risk of this pathway of microplastics through the food chain and understand the factors affecting the risk so that proper mitigative and preventive measures can be taken to reduce or eliminate the risk to animals, humans, and the environment. Polar cod is an important part of the ecosystem. They reside in the Arctic, where concentrations are high, they rely on organisms that are in the lower food chain, and they are consumed by upper levels and play a critical role in the entire food chain.

## 1.2. Polar cod

Aquatic life is susceptible to illness and even death from microplastic exposure (Wright et al., 2013). Polar cod, or *Boreogadus saida*, is a circumpolar marine fish found in abundance in the fast-changing Arctic ecosystem. They produce antifreeze glycoproteins which help them adapt to subfreezing temperatures (Osuga & Feeney, 1978). Polar cod is the most vital species of the short and simple Arctic food chain. It has a strong relationship at every trophic level. Polar cod is a forage fish and preys upon lower trophic species like krill, copepods, amphipods and other arctic zooplanktons, and is in turn preyed upon by higher trophic species such as polar bears, seals, birds, beluga whales, and Arctic fox. Seals, polar bears, walrus and many avian species rely primarily on polar cod for their survival. It is to the credit of polar cod that in the high arctic food web, almost three-quarters of the zooplankton production is channeled through them to the higher predatory species (Bakke et al., 2016; Benoit et al., 2014; Hop & Gjøsæter, 2013a). This unique positioning of polar cod in the Arctic food web makes it an excellent selection for this study. Many studies like (Christiansen et al., 2014; Tomy et al., 2014) have also identified polar cod to be an excellent indicator of the associated risk in the food chain.

The knowledge gaps or challenges in the ecotoxicological risk assessment of microplastics in the Arctic region are:

- 1) Paucity of literature on microplastics toxicity modelling, particularly in ice-infested Arctic waters. Ice cover traps the microplastics. Thus, MPs intake varies greatly from one species to another. Species like seals take food from ice-cover regions; thus, they are overexposed, while others like capelin are less exposed.
- 2) Lack of reference data – Reference values like Chronic Reference Dose (RfD), RBSLs, SSTLs value for microplastics are not available. Also, toxicity data on Arctic species is rare. The lack of data is generally compensated by using the data of temperate species. However, the practice is highly questionable owing to the many environmental, and geophysical distinctions in both the regions and physiological differences between the species.
- 3) Behaviour of Arctic species and food chain features: the Arctic food chain has some interesting features. Many species rely mostly on one prey to meet their energy requirements. For instance, whales mostly eat small fish, like polar cod, whereas polar bears' diet is mainly composed of seals. This feature of the aquatic food chain has a cascading effect on the next trophic level (Nevalainen et al., 2017).

This study aims to overcome these limitations by developing a comprehensive ecotoxicity risk assessment model for polar cod exposed to microplastics in the Arctic region. It appraises the likelihood of cell death in polar cod after microplastic ingestion. The developed model circumvents the traditional use of toxicity assays by adopting a Bayesian-based approach that projects the probability of cell death in the cod after ingestion. The study aims to graphically represent the Toxicokinetic (TK) and Toxicodynamic (TD) processes in polar cod. Various environmental, geophysical, and physiological parameters influencing the TK and TD processes in polar cod are identified. A cause-effect relation is then established between these parameters to develop a BN. This complex BN is then transformed into an Object-Oriented Bayesian Network (OOBN) for simplification to determine the cell death likelihood, which further studies can subsequently use to evaluate the organism fatality.

## 2. Toxicokinetic (TK) mechanism

Toxicokinetics (TK) describes how species respond to toxicants inside their bodies. It covers the overall description via four key concepts, i.e., absorption, distribution, metabolism, and elimination (ADME). The contaminant is absorbed and distributed in the whole body via systemic circulation. It gets metabolized in the liver and then eliminated via feces and gills. TK models quantize the xenobiotic distribution across the various organs in the species.

### 2.1. Absorption and distribution

Microplastic, owing to its small size, finds its way into amphipods, copepods and other zooplanktons easily (Cole et al., 2013). These zooplanktons are eaten by polar cod. Moreover, while foraging, polar cod often mistakenly consume microplastics particles. Microplastic intake is

**Table 1**  
Nodes of BN, their states and description.

Nodes	States	Description	References
Season	Winter	Winter spans from December to June (0.58)	
	Summer	Summer spans from July to September (0.25)	
	Autumn	Autumn spans from October to November (0.17)	
Arctic ice thickness	Low	Ice thickness in the Fram Strait in Arctic region varies from 0.8 m to 3.5 m.	
	Medium	Low – 0.8–1.5 m Med – 1.5–2.5 m High – 2.5–3.5 m	
	High		
Salinity	Low	Salinity increases with drop in temperature. As ice forms at the top, it pushes the salts to the bottom.	
	Medium	Low – < 10% Med – < 25% High – < 40%	
	High		
Microplastic Particle Size	Low	MP size ranges from 1 µm to 5 mm	
	High	Low – < 2.5 mm (0.65) High – 2.5–5 mm (0.35)	
Microplastic Particle Density	Low	MPs are present throughout the water column in the Arctic. They decrease as we go down the water column before increasing drastically at the sediments.	
	Medium	0.012 N/m <sup>3</sup> to 0.144 N/m <sup>3</sup> when measured using 335 µm mesh.	
	High	9 N/m <sup>3</sup> to 1287 N/m <sup>3</sup> when measured using 32 µm mesh. 4356 MP pieces weigh 1 kg in the Fram Strait. Low – < 700 N/m <sup>3</sup> or <161 gm/m <sup>3</sup> (0.25) High – 700–1300 N/m <sup>3</sup> or 161–298 g/m <sup>3</sup> (0.75)	
Feeding activity	Low	Feeding is high during autumn and summer and low during winter.	
	Medium	Low – < 15% High – > 15%	
	High		
MP lipid accumulation	Low	The concentration of MP and associated toxicants are trapped in the lipids, particularly in liver lipids, expressed as percentages.	
	High	Low – < 25% High – < 25%	
MP Bioavailable concentration	Low	Gills remove major portion of toxicants. The states in the node are defined as:	
	High	Low – < 70% High – > 70%	
MP liver Concentration	Low	More than three-quarter of the xenobiotic intake in the fish is metabolized in the liver. The states in the node are defined as follows:	
	High	Low – < 70% High – > 70%	
Liver microsomes	Low	About 15–20% of the liver area is taken by endoplasmic reticulum. The states of the node are defined as:	
	High	Low – 5–12% (0.35) High – 12–25% (0.65)	

**Table 1 (continued)**

Nodes	States	Description	References
Baseline Phase I activity	Normal	The baseline phase I activity ranges from 3 to 13 pmol/min/mg.	Rodd et al. (2017)
	Elevated	Normal < 8 pmol/min/mg Elevated > 8 pmol/min/mg	
Baseline Phase II activity	Normal	The baseline phase II ranges from 200 to 250 pmol/min/mg.	Nahrgang et al. (2010)
	Elevated	Normal < 350 pmol/min/mg Elevated > 350 pmol/min/mg	
Phase I activity	Low	The phase I activity is measured increase in folds of the EROD activity (Ethoxresorufin-O-deethylase)	Rodd et al. (2017)
	High	Low – ≤ 8 High – ≥ 10	
Phase II activity	Low	The Phase II is measured in fold increase in GST activity (Glutathione S-transferase)	Nahrgang et al. (2010)
	High	Low – ≤ 4 High – ≥ 7	
Cell damage from biotransformation	Low	Low – 5–15% High – 15–30%	Expert Opinion

also observed via water ingestion (Kohlbach et al., 2017; Lonne & Gulliksen, 1989). The ingested microplastics reach the liver, from where they are distributed to the whole body through the systemic circulation. Fig. 2 depicts the route of microplastic in polar cod after oral ingestion.

## 2.2. Biotransformation and elimination

Microplastics reach the liver of the polar cod through the intestine, where it is acted upon by enzymes, causing biotransformation. From the liver, some part of it reaches the systemic circulation, which also leaks small amounts to fats and other depositions. Biotransformation occurs in the liver in two phases. In phase I, the CYP1 group of enzymes acts on the microplastic xenobiotics to form water-soluble metabolites. The phase I process is characterized by the addition of polar atoms to the xenobiotics to form hydrophilic metabolites that are either easily eliminated or result in toxification or inert presence in the body (Santana et al., 2018). Oxidation is concomitant to processes like biotransformation. Hence, a balance should be there to counter its negative effects. This is what occurs in phase II reactions. The phase II process is a conjugation reaction of the oxidized metabolite with glutathione (GSH) and is facilitated by the Glutathione S-transferases (GST) enzyme, thereby acting as an antioxidant defence in cod (Giulio & Hinton, 2008).

Metabolites formed because of phase I reactions lead to cytotoxicity if they are not conjugated by the phase II process. So, cell death occurs when the concentration of toxic metabolites produced after phase I reaction exceeds the conjugating capacity (Banni et al., 2009). Cells, however, possess an innate ability to repair themselves but only up to a threshold. If a greater number of cells than the threshold dies, the organ fails. Apart from the oxidative stress due to biotransformation, peroxidation of lipids is also a cytotoxicity mechanism. However, it is not considered in our analysis. To quantify the metabolite concentration in polar cod, Ethoxresorufin-O-deethylase activity (EROD) assay is used as a phase I biomarker while glutathione-S-transferase (GST) assay is used as a phase II biomarker.

The metabolized microplastics are then eliminated via the hepatobiliary tract. Gills also act as another source of xenobiotic removal, but this is not considered in this study (Fig. 3).

### Phase I mechanism:

The microplastics ingested by polar cod get oxidized in the presence of Cytochrome P450 oxidase. Polar groups get attached to them to form

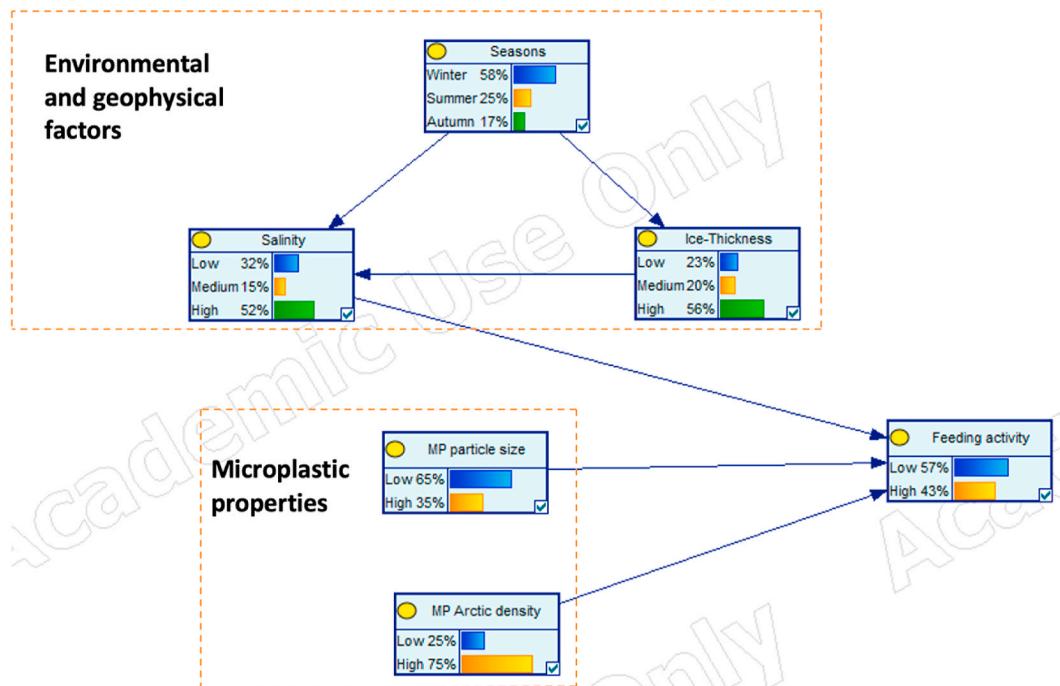


Fig. 6. Feeding Activity instance node of the developed OOBN.

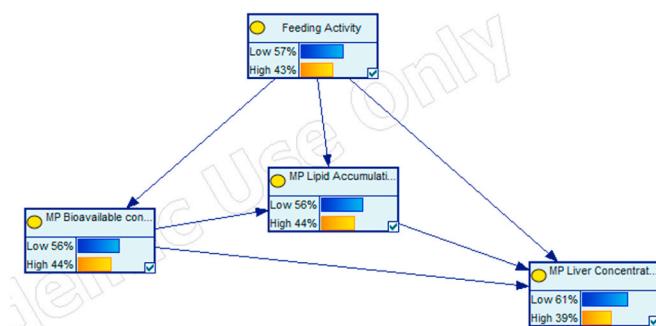
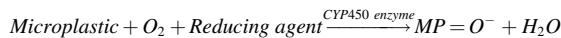


Fig. 7. Microplastic concentration reaching liver instance node of the developed OOBN.

reactionary intermediary metabolites.



#### Phase II mechanism:

The reactionary intermediary metabolites produced in the phase I mechanism are now acted upon by glutathione in the presence of the GST enzyme. If the GST activity is high, all of the reactionary intermediary metabolites are detoxified to glutathione conjugated metabolites. If the GST activity is low, superoxide ( $\text{O}_2^-$ ) is produced, which is converted to  $\text{H}_2\text{O}_2$  via SOD enzyme activity and eventually to  $\text{OH}^-$  by the Fenton reaction. This damages cell DNA and also leads to lipid peroxidation, resulting in cell death.

### 2.3. Deleterious effects of microplastic intake

Studies by (Alomar et al., 2021; Choi et al., 2022; Maaghlood et al., 2021; Nanninga et al., 2020; Rist et al., 2020) have investigated microplastic ingestion on various zooplanktons and fish. Microplastic intake results in reduced feeding behaviour, false satiation sense, growth inhibition and fecundity inhibition in polar cod. Microplastic debris blocks the intestinal tract, which causes injury to its internal system and

pseudo-satiation sense (Kühn et al., 2018). Very fine microplastic particles pave their way to cells and organs, with consequences still under research (Brennecke et al., 2016; Browne et al., 2008). (Sun et al., 2016) shows the presence of various absorbed persistent organic pollutants on the deployed samples of polyethylene in western Svalbard. The adsorbed chemicals are transferred to the organisms upon consumption, which leads to many health problems (Chen et al., 2018).

### 3. Ecological risk methodology and application

The steps in the methodology, as shown in Fig. 4, are as follows:

- 1) Identify factors (environmental, geophysical, physiological factors and microplastic properties) affecting microplastic intake and distribution in the polar cod.
- 2) Identify the temporal (seasonal) changes in the enzymatic activity affecting the microplastic toxicity to polar cod.
- 3) Determine the baseline values of the identified environmental, geophysical, physiological factors and microplastic properties.
- 4) Identify the cause-and-effect relationship between the identified factors.
- 5) Develop the structure of a Bayesian network using these identified factors.
- 6) Collate data from the available literature and expert elucidation.
- 7) Use Object Oriented BN to simplify the BN for better realization.
- 8) Determine changes in biomarker values due to microplastic intake.
- 9) Determine the cell death probability from the developed BN model.

These steps offer an ecological risk model aimed at determining the likelihood of cell fatality due to microplastic intake in polar cod using the cause-and-effect relationships established in the Bayesian network.

#### 3.1. Factors affecting microplastic intake and distribution in polar cod

There are three categories of factors that determine the microplastic intake in polar cod. The first one is the region-specific environmental and geophysical factors. Another is physiological factors of the polar cod, and the last one is the properties of the microplastic particle. The

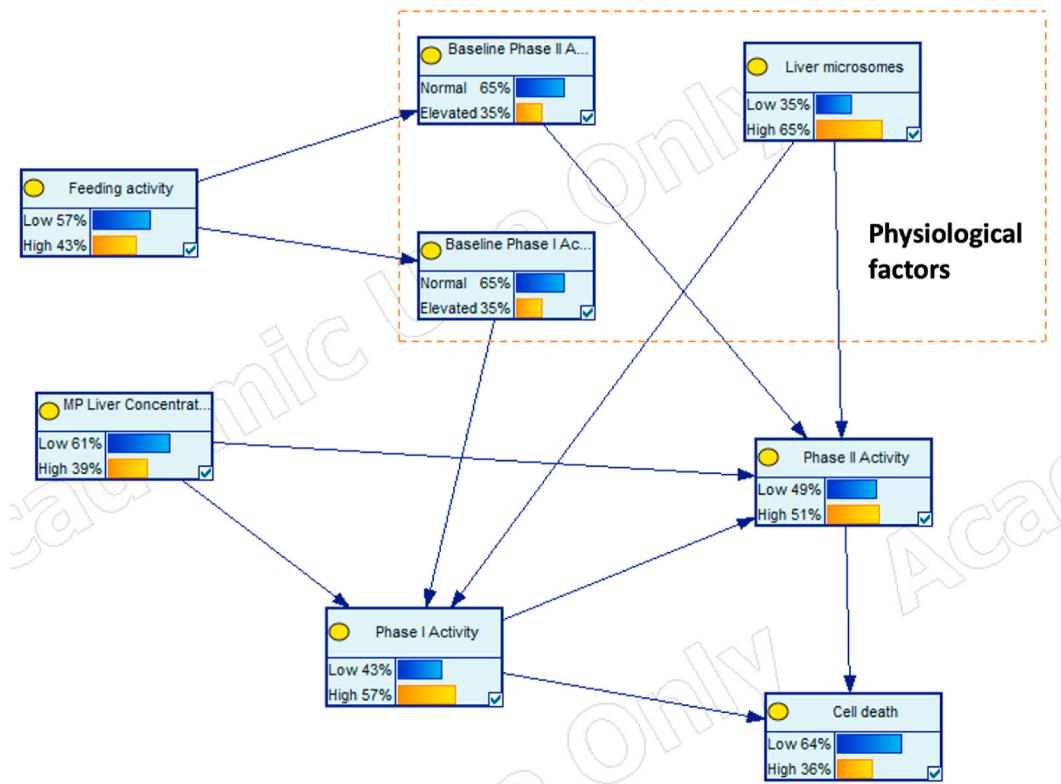


Fig. 8. Cell death instance node of the developed OOBN.

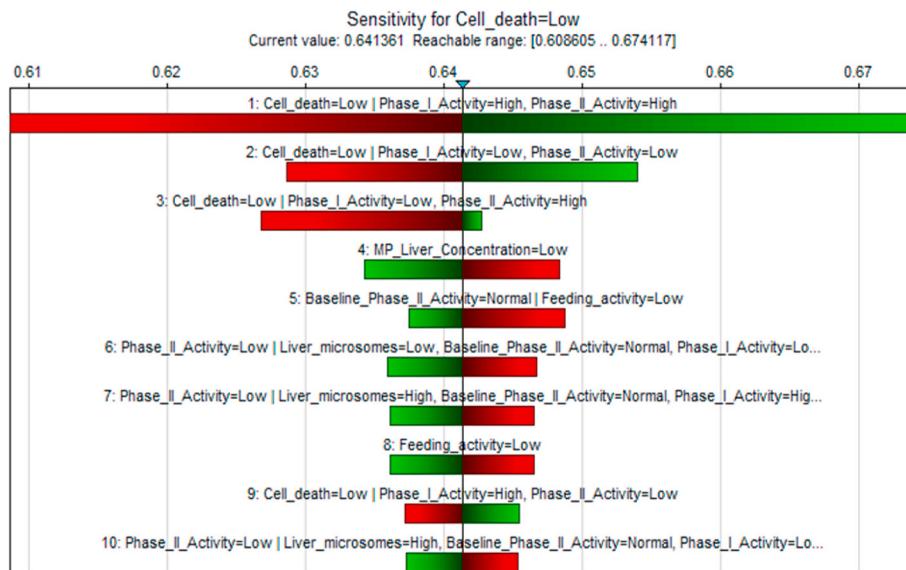


Fig. 9. Sensitivity tornado for cell death.

Arctic region is uniquely characterized by harsh weather conditions, the presence of a thick ice layer and an extreme light regime, including periods of midnight sun and polar night (Berge et al., 2015). Along with these unique physical characteristics, the transfer of energy along the various trophic levels of the food chain is also idiosyncratic (Werner, 2006). Thus, the environmental and geophysical factors identified are season, sea-ice thickness, and salinity. The polar cod's physiological factors are liver microsomes and baseline enzymatic activity in both the phases. Also, properties of microplastics like their size and density influence their intake and distribution.

### 3.1.1. Environmental and geophysical factors in the arctic region

The Arctic undergoes three seasons, namely, winter, summer, and autumn. In winter, ice-thickness increases, which in turn increases the salinity as the formed ice pushes the brine deep into the seawater. Ice-melt and lower salinity in summer facilitate the increased availability of sunlight in the ocean, which enhances the algal activity. Algae are consumed by amphipods and copepods, which are then eaten by polar cod, thereby increasing the feeding activity (Berge et al., 2015).

### 3.1.2. Microplastic particle properties

The properties of microplastic, like its shape, size, density, and composition, influence its intake by all species in general and polar cod. For this study, microplastic size and density are considered. Lower microplastic size and higher density lead to higher feeding activity (Rist et al., 2020). noted that the concentration of microplastic increases with decreasing particle size.

### 3.1.3. Physiological factors of the polar cod

Physiological parameters like liver microsomes and the baseline enzymatic activities affect the biotransformation, cell response and distribution. Around one-fifth of the cell area is the smooth endoplasmic reticulum, where phase I CYP1A enzymes are present. Approximately 2% of the cytosolic protein in polar cod are phase II activity enzymes (Moore, 1992).

The elevated baseline enzymatic activity enhances the corresponding phase activity, which eventually determines the cell damage probability.

## 3.2. Temporal (seasonal) changes in enzymatic activity

Enzymatic activity of polar cod is directly contingent on food availability. During the summer season, a reasonable amount of sunlight enters the Arctic ecosystem. This sets the phytoplankton in action through photosynthesis and thus energy comes into the ecosystem. This is less prominent during the winter season. Thus, a feeding outburst is observed in the summer season, which leads to fat reserves in polar cod. These fat reserves are crucial during spawning in the following months of early winter (Hop & Gjøsæter, 2013b). Hence, enzymatic activities are enhanced during the summer season and gradually decrease as the feeding activity changes.

## 3.3. Baseline data of environmental, geophysical and physiological factors

The Arctic region experiences four seasons, of which three are considered in this study, namely winter, summer, and autumn. Winter lasts the longest, from December to June, followed by summer from July to September and the remaining months of October and November are autumn. So, the prior probabilities of winter, summer, and autumn are 0.58, 0.25 and 0.17 respectively.

The data of ice-thickness are taken from Fram Strait (Werner, 2006) and the Kongsfjord region (Nahrgang et al., 2010). The only deep-water connection between the Arctic and Atlantic is Fram Strait, which lies between Svalbard and Greenland (Thiede et al., 1990). The ice thickness varies between 0.8 m and 3.5 m throughout the year. Ocean salinity ranges from 16.7 psu to 34.6 psu.

Liver microsomes are taken to vary from 5% to 25% in polar cod. The baseline phase I activity is measured in increase in folds of the CYP1A enzyme activity and the baseline phase II activity is measured in increase in folds of the GST activity. Baseline phase I activity and baseline phase II activity range from 3 to 13 pmol/min/mg to 200–250 pmol/min/mg respectively. (Nahrgang et al., 2010; Rodd et al., 2017).

Plastic deposition in the Fram Strait has exhibited a meteoric rise during the last few years (Martínez et al., 2020). Previous studies have also established that Fram Strait has the highest microplastic concentration in the whole Arctic and that the size of most particles is smaller than 50 µm (Peeken et al., 2018). The studies (Hänninen et al., 2021; Tekman et al., 2020) have shown the presence of microplastic across the Arctic water column, with their values ranging from 0.012 N/m<sup>3</sup> to 1287 N/m<sup>3</sup>. Also, they come in all sizes, from 5 mm to 1 µm.

## 3.4. Develop an OOBN using available literature and expert elucidation

Bayesian networks (BN) are directed acyclic graphs (DAGs) containing nodes and arcs that satisfy the Markovian condition. A BN uses known quantitative information to determine the posterior probability.

When there are many nodes in the BN, it becomes visually unpleasant and difficult to understand. In such a case, an Object-oriented Bayesian network (OOBN) is developed as a hierarchy of sub-networks with desired abstraction levels to simplify the otherwise complex BN (Kjaerulf & Madsen, 2008). Similar usual nodes are clubbed together as one instance node, which is then the input of the next sub-network (Fig. 5). Table 1 lists all the nodes and assigns a range of values characterizing the nodes.

The instance nodes of the object-oriented Bayesian network of the study conducted are in Fig. 6, Fig. 7, and Fig. 8 below.

## 3.5. Model testing and benchmarking

The OOBN-based ecotoxicity model is tested using a study by Mahadevan & Valiyaveettil (2021). The study assesses the impact on BHK-21 cells or baby hamster kidney cells on being exposed to very small plastic particles of polyvinyl chloride (PVC) and polymethyl methacrylate (PMMA). The induced cellular biochemical changes in BHK-21 cells like cell morphology, cell fatality and concentrations of reactive oxygen species (ROS) were monitored at various concentrations of the plastic dose over a period of time. Results exhibit a substantial decrease in cell viability, or in other words, an increase in cell death, at  $40.3 \pm 0.1\%$  for PVC and  $61.3 \pm 4.0\%$  for PMMA when 200 µg/mL is the exposed concentration for 120 h. The developed OOBN model also shows similar results. In the model, high feeding activity is an outcome of high microplastic concentration, which is defined as 161–298 g/m<sup>3</sup> or 161–298 µg/mL. This means that when the exposed concentration is 200 µg/mL, cell death is high.

## 4. Results and discussion

Seasonal variations in the Arctic region affect the sea ice thickness and water salinity. The latter is influenced by the former, and the microplastic properties like size and density shape most species' absorption or feeding activity, including polar cod. Feeding activity determines the xenobiotic distribution in the systemic circulation and lipids, which collectively decides the pollutants' concentration reaching the liver. Feeding activity induces changes in baseline enzymatic activity, which affects phase I and phase II activities. Xenobiotic concentration in the liver and physiological factors like liver microsomes and baseline enzymatic activities also influence phase I and phase II activities. The developed model identifies the causal dependencies between all the associated variables to determine the outcome in the form of cell death likelihood.

The cell death likelihood gives an idea of the toxicity of microplastics to polar cod and can be used to assess organ failure and organism fatality further. It can also be extended to estimate the impact across the Arctic food chain. In GeNie software by BayesFusion LLC, sensitivity analysis of the node 'cell death' is performed (Fig. 9). Sensitivity analysis is done to identify the most important factors that affect cell death. The results show that the phase I and phase II activities have the highest effect on cell death, followed by the concentration of microplastics reaching the liver and feeding activity.

The unchecked plastic waste is a serious threat to the Arctic ecosystem. The proposed model presents a stochastic approach to estimate the microplastic toxicity in polar cod without toxicity assays. This is achieved by identifying the various factors that influence the TK and TD processes in polar cod, establishing the cause effect relationship between them and then developing a Bayesian belief network to estimate the cell death likelihood. For clarity, the BN is simplified into OOBN. The model shows how the microplastic in the Arctic region facilitates cell fatality in polar cod, which will eventually lead to organ failure and organism death. The sensitivity analysis further identifies the most crucial factors which impede cell viability.

The study, however, is not free from limitations. It assumes the temporal static nature of the ice-thickness and salinity within a season, i.e.

e., variations within a season are not considered. Data available from literature and expert opinions were considered for the probabilities of various nodes of the Bayesian Network. Also, some assumptions were made in the conditional probabilities of the Bayesian Network. **Giulio & Hinton (2008)** detail the various pathways of biotransformation, which are very complex, and subjected to numerous factors. For the sake of simplicity, only the major pathway, i.e., CYP1A, is considered in this study.

In future, as more information is available, the probabilities in the BN model can be updated to get better results. Additionally, a time variable can be incorporated for more accurate risk probabilities. Moreover, extending the probability of cell death to organism fatality is another challenge for future work. Also, the organism fatality, in turn, can be extrapolated using a suitable population model to develop an understanding of the health of colonies of species. While considering other possible biotransformation pathways, the phase I and phase II activity should be replaced by relevant biomarkers. Cell death from lipid peroxidation and DNA damage due to biotransformation can also be considered using suitable biomarkers. Biomarkers sensitive to phase I and phase II activities, in addition to EROD and GST after identification, can be incorporated into the developed model. Biomarkers for other biotransformation pathways, once identified, can also be added to the model to produce more accurate results. As new information and data in the field of genomics become available, they can be considered in the model to estimate more precise trends in polar cod.

## 5. Conclusions

The present study proposes a comprehensive model to assess the risk posed by microplastics to polar cod in the Arctic environment. The physiological factors of the polar cod and the environmental and geophysical factors of the region affecting microplastic intake, distribution, and biotransformation in polar cod are considered. A cause-effect relationship between all the factors is established to develop an Object-Oriented Bayesian Network that determines the cytotoxicity. The biotransformation of microplastics and their metabolites results in oxidative stress, which leads to cytotoxicity. On being exposed to microplastics, changes in cell metabolic activity and antioxidant defence activity are measured using Ethoxresorufin-O-demethylase activity (EROD) assay and glutathione-S-transferase (GST) as biomarkers.

The traditional approach, which is used extensively to measure the toxicity of a xenobiotic to a temperate fish, is often challenged for Arctic species. This is because it fails to encompass the *sui generis* features of the Arctic region. The developed model addresses this issue effectively by creating an all-encompassing and holistic model using a Bayesian Network. The model evaluates changes in the homeostatic functioning of polar cod by predicting cell toxicity.

The risk deemed in this study is the death of a cell in polar cod. This occurs when the phase I activity outcome (ROS) is not conjugated by phase II activity. **Fahd et al. (2020)** propose that when 35% of cells in an organ die, a fatality occurs. Using this observation and the OOBN presented in the current study, a detailed stochastic ecological risk model for microplastic is developed. The developed model will serve as an essential tool to establish arctic risk management policies and strategies.

## Authorship statement

**Mohammad Sadiq Saeed:** Conceptualization, Methodology, Formal Analysis, Investigation, Writing - Original Draft; Writing - Review & Editing. **Syeda Zohra Halim:** Methodology, Formal Analysis, Writing - Review & Editing; **Faisal Fahd:** Methodology, Formal Analysis, Writing - Review & Editing; **Faisal Khan:** Conceptualization, Methodology, Writing - Review & Editing; Supervision; Project administration; Funding acquisition. **Rehan Sadiq:** Conceptualization, Methodology, Formal Analysis, Writing - Review & Editing; Supervision; Funding acquisition. **Bing Chen:** Conceptualization, Methodology, Formal Analysis, Writing -

Review & Editing; Supervision; Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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