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## Review

## Diversity of bacteria and fungi associated with tarballs: Recent developments and future prospects

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## ABSTRACT

Tarballs are formed by weathering of crude oil in marine environment. They are transported from open ocean to the shores by sea currents and waves. Tarball pollution is a major concern to global marine ecosystem. Microbes such as bacteria and fungi are known to be associated with tarballs. They presumably play an important role in tarball degradation and some are potential human and animal pathogens. This paper highlights the recent studies on tarball-associated bacteria and fungi. Future perspectives on diversity, ecology and possible applications of tarball-associated microbes in bioremediation of beached tarballs have been discussed.

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## 1. Formation of tarballs in marine environment

Fossil fuels are precious, non-renewable energy sources. There has been a steady growth in the consumption of petroleum and its products all over the world (US Energy Information Administration, 2016). As most of oil transport is done *via* sea, occasional accidental spillages result in the introduction of crude oil into marine environment. However, crude oil can also enter marine environment through release of ballast water from ships, operational discharges during offshore drilling,

pipeline ruptures and natural seepage from the seabed (Harayama et al., 1999; Das and Chandran, 2011). It is estimated that the oceans receive 0.47–8.40 million tonnes of oil (petroleum hydrocarbons) annually (Global Marine Oil Pollution Information Gateway, <http://oils.gpa.unep.org/facts/quantities.htm>) and some of it ends up on the shores as tarballs.

Tarballs are formed when the crude oil released into marine environment by anthropogenic or natural activities changes over time due to weathering (Chandru et al., 2008). The weathering involves various physical, chemical and biological processes. The physical processes include spreading, evaporation, dispersion, dissolution, sedimentation and emulsification. The chemical and biological processes include photo-oxidation or photo-degradation and biodegradation (Harayama

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et al., 1999; Wang et al., 2011), respectively. Though the exact mechanisms of tarball formation are poorly understood, the weathering processes are presumed to occur simultaneously. The weathering is believed to depend on environmental conditions (such as temperature, wind velocity, ocean current dynamics, and dissolved oxygen concentration), type and source of oil, and time and amount of oil spillage (Jordan and Payne, 1980). Chandru et al. (2008) provided a detailed explanation on tarball formation.

Tarballs have solid or semi-solid consistency. Their size varies from a few millimeter to several centimeters, while the color ranges from black to brown (Fig. 1). Their texture can be brittle, hard or soft and very sticky, depending on their formation conditions (Chandru et al., 2008; Wang et al., 2011). Some tarballs have a uniform structure throughout, while others may be solid externally and liquid internally (Goodman, 2003). Recently, Warnock et al. (2015) have reviewed the research conducted on marine tars since 1970s, including formation, distribution, transportation, chemical composition, source tracking and degradation of tarballs. The geographical locations of tarball pollution at the global level are presented in Fig. 2.

Tarballs chemically differ from their parent crude oil, depending on the formation conditions (Wang et al., 1998). For example, if a weathering process became dominant, it could influence the composition of resulting tarballs (Chandru et al., 2008). During weathering, if oxygen from the atmosphere gets mixed with hydrocarbons present in the crude oil, it can form oxygenated hydrocarbons (“oxyhydrocarbons”). Oxyhydrocarbons are known to be recalcitrant in nature (Kimes et al., 2014). It has been reported that heavy hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) are enriched in tarballs after lighter hydrocarbon fractions dissolve/evaporate during weathering (Harayama et al., 1999; Nemirovskaya, 2011). Meta-toluic acid, a toxic compound present in tarballs, is known to persist in marine environment (Prakash et al., 2008).

Tarballs have high metal concentration than the parent crude oil. It is quite possible that high molecular compounds present in weathering crude oil act as chelating groups on which metal ions bind from sea water (Liu et al., 2012). Metals such as vanadium, nickel, copper, iron, magnesium and cobalt have been reported from tarballs (Wong et al., 1976; Hegazi, 2009; Zare-maivan, 2010; Liu et al., 2012) but their concentration varies across different reports. Metals present in tarballs can facilitate transformation of hydrocarbons through oxidation, resulting in environmentally persistent free radicals (Kimes et al., 2014). It has been suggested that increased concentration of metals enhances the toxicity of hydrocarbons and affects microbial activity (Shukla and Cameotra, 2012).

## 2. Consequences of tarball pollution

The adverse effects of tarballs on environment are poorly understood as compared to pollution caused by fresh oil spillage. Available studies suggest that weathered oil is less toxic than the crude oil (Warnock et al., 2015). Newly formed tarballs are considered to be

more toxic than older or highly weathered one's, because the former contains abundant water soluble and low molecular weight compounds (hydrocarbons) which are considered to be more toxic in nature (Harayama et al., 1999). Tarball pollution can result in economic and ecological losses. For example, Goa state located on the West Coast of India is a global tourist destination. Tarballs deposited on Goan beaches are likely to negatively affect the coastal ecology and local economy (Rekadwad and Khobragade, 2015). Sea water that gets contaminated with tarballs has petroleum-like odor, making it less-suitable for swimmers. Tarballs pose a high risk to human health as some individuals can be allergic to tarballs (<http://www.emaxhealth.com/1275/bp-oil-spill-dangerous-childrens-lungs>). Tarballs that travel towards the coast can stick to the fishing nets installed in the sea by fishermen, making them difficult to clean.

Crude oil contains chemically diverse hydrocarbons, including PAHs. Tarballs, as residual crude oil, are expected to contain abundant carcinogenic PAHs, which may affect humans and marine organisms on long-term exposure (Shukla and Cameotra, 2012). Tarball surface serves as a substrate for bacteria, fungi, unicellular algae and other microbes (Nair et al., 1972; Tao et al., 2011). Tao et al. (2011) analyzed tarballs for total aerobic bacterial counts and for the presence of *Vibrio vulnificus*, a human pathogen. It was observed that total bacterial counts were higher in tarballs as compared to those in sand and sea water. The presence of *V. vulnificus* was 10 and 100 times higher in tarballs as compared to sand and sea water, respectively. It is not clear why tarball surface supports high concentration of microbes. It is believed that microbes feed on the by-products formed by hydrocarbon degrading bacteria present in/on tarballs (Tao et al., 2011).

Animals and plants may die because of exposure to fumes released by tarballs. Sea animals such as turtles can accidentally consume tarballs as food, leading to their death (Goodman, 2003; Warnock et al., 2015). Horn et al. (1970) reported tar residues from stomach of an epipelagic fish, *Scorpaenopsis saurus* and stated that the toxic components of tarballs can easily enter into food chain. Tarballs become heavier after being mixed with sand and sink to the bottom of sea, disturbing living conditions of benthic organisms, including nursing grounds of fish and shellfish (<http://oils.gpa.unep.org/facts/habitats.htm>). The toxic contents of less-weathered tarballs can be lethal to marine organisms such as bivalves, shrimps, which serve as food for pelagic fishes, leading to decrease in food to the next level animals in the food chain, and thus indirectly affecting fisheries (<http://timesofindia.indiatimes.com/india/Tar-balls-hit-beaches-fauna-and-Goa-tourism/articleshow/37675282.cms>). Parish government reported more than 12 m land loss of salt marshes by suffocation caused by tar-like clumps (Bruckner, 2011). In addition, the cleaning drives of tarballs using heavy equipment can disturb marine niches.

## 3. Microbial diversity associated with tarballs

Table 1 presents a list of bacterial and fungal taxa reported till date from tarballs. In early 1970s, microbes such as blue-green algae,

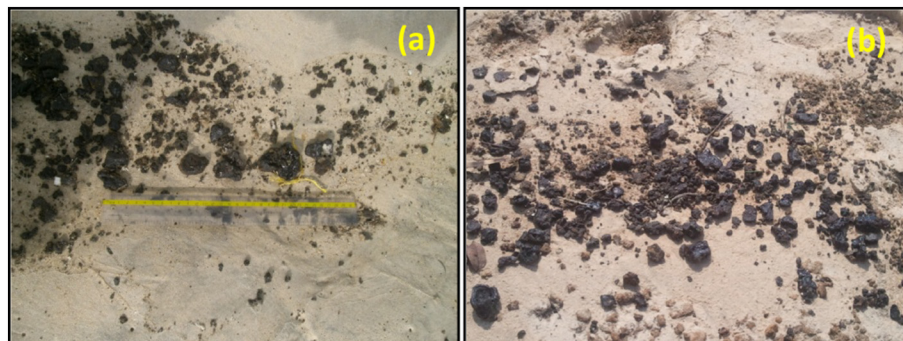


Fig. 1. Tarball deposits on beaches of Goa, India, a: Benaullim beach, b: Candolim beach.

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