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Adverse Effects of Mariculture Activities and Practices on Marine Environment

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Introduction

Aquaculture activities are increasing worldwide due to the rapid increase in world population and increase in demand for aquaculture products. In recent years, interest of consumers on aquaculture products such as fish and arthropod increased in EU, there by fish farming rapidly increased, in contrast, natural fish stocks were decreased [1]. Increased demand on fish mainly satisfied by salmon (*Salmo salar*) in cold regions, by sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) in Mediterranean and warm regions [2-4]. Growing demands of the consumers will be satisfied along with adaptive aquaculture practices which reduce dependence on natural stocks. It will also create a new business area for the people who live in coastal areas and reduce the economic impacts on them [5].

Over the last decade, aquaculture industry is growing continuously hereat the environmental effects of the aquaculture activities are reached remarkable size. Although this increase in aquaculture industry provides economic benefits, surrounding aquatic environment is getting more polluted biologically, physically and chemically also changing appearance of natural environment. It is hard to distinguish the real and potential effects of the aquaculture or determine the enormity. However, the real thing is, impacts of aquaculture activities cover many problems from aesthetic appearance to direct pollution [6-8]. The aquaculture activities in the sea and the facilities that provide activity disrupt the natural appearance. Seas are getting polluted by direct waste disposal and by chemical use. Impacts of the discarded waste in water column depend on geographical features of the regions and bottom features of the sea. Dissolved constituents (ammonium, phosphorus and nitrogen dissolved organic carbons), oils and lipids released from the diet can be listed as some of the discarded wastes.

Currently, only 30% of the cultured fish, which are herbivore fish species (such as cyprinids),are produced without feed or with limited feed usage compared to 50% in 1980s [9,10]. Increase in aquaculture activities along with demand arising from wealthy populations, channeled aquaculture toward carnivorous fish species which rely on feed. As a result, the main material released to the water column becomes fish feed. While some of the feed is converted into proteins by fish, some of them released into water column as dissolved substances such as carbon, nitrogen, phosphate. Consumed feed wastes are excreted through kidney and gills to water body as well (undigested substances like ammonium, urea etc.). Moreover, when drugs are used for disease treatment or prevention of diseases, and are released to the water column, undesired negative effects on the environment and organisms are inevitable [11-14]. Genetic disorders [15], pathogen carrier cultured fish escapes from cages or digestion of contaminated wastes causeundesired changes on natural populations and directly affecting the ecosystem [16].

Impacts of Mari Culture on Water Quality

Effects of waste on water body depend on the key features of the habitat, waste quantity, time-scale over which the releases take place and characteristics of the water column. Aquaculture originated wastes can be subdivided into two major groups; solid waste and soluble waste. Solid wastes are composed of uneaten pellet feed, faeces, fish scale and mucus. Since most of them are denser than water, they sink to the bottom. Soluble wastes are ammonium, urea and some of the particles that solved from solid wastes. Among these wastes, nitrogen, phosphate groups and suspended substances are the main components that affect water quality. Moreover, some of the vitamins and minerals (trace elements) are effective on algae populations [17]. Aquaculture originated pollution is generally divided into two categories as organic and chemical pollution.

Organic Pollution

The main sources of organic pollutants are fish faeces, uneaten feed and dead individuals. Faeces produced by fish are related with the feed ingredients. 25-50% of the consumed feeds are excreted to environment as faeces. Intensive fish farming causes large amounts of organic waste accumulation in the sediments and water column [18,19]. A sediment collector placed under salmon cultivation cages collects 14.7-52 kg of organic waste in each square meter per annum, which reveals the extent of organic pollution originating from aquaculture activities. Aquaculture activities lead a number of ecological issues. For instance, aquaculture facilities such as cage systems significantly reduce water current speed thus cause accumulation of organic substances in surrounding environment. Decomposition of these organic wastes lead deterioration of water quality and increase water oxygen requirement. Negative effects like turbidity, accumulation of organic waste in the sediment, formation of anoxic zone near sea floor, accumulation of toxic substances and spread of diseases are influential in areas where aquaculture is in practice, especially in restricted exchange environments.

Formation of gasses such as H_2S (hydrogen sulfide), CH_4 (Methane) and CO_2 (Carbon dioxide) on sediment slows down growth rate of living organisms in the local area and makes them more vulnerable to diseases [20]. Water oxygen level is reduced by direct decomposition of organic waste originated from aquaculture activities. Water quality changes by releasing nutrients like nitrogen and phosphate into the water column. Excessive amount of organic nutrients promote growth of water plants and algae. When toxin producing algae grow excessively, they may reach blooming concentrations and cause negative impacts to other organisms. Cultured fish may die off due to the disorders occurring in the gills and oxygen deficiency as a result of water quality changes related with sudden growth of algae. Consumers of bio toxin contaminated shellfish can get poisoned and die as well [21]. When algae die in large numbers, dead organic substances start to decay and this process consumes oxygen, leads to hypoxia. Accumulation of organic and inorganic wastes in areas with low water exchange rate may lead hyper-nitrification as well.

In this sense, permanent aquaculture activities in such regions accelerate accumulation of waste. On the other hand, aquaculture activities held in deep waters or areas with high bottom current rate may result in wide spread of wastes. Let's make a comparison to explain extend of pollution caused by aquaculture activities. An average person excretes 4kg of nitrogen and 1.1kg of phosphate per year. For every ton of salmon 55kg of nitrogen and 4.8kg of phosphate are released into the water column through faeces per year. A salmon cultivation facility with a production capacity of50000 tons a year release an amount of nitrogen equals to that of 682000 people, and an amount of phosphate equals to that of 216000 people into the water in a year.

Eutrophication

Eutrophication simply means enhanced nourishment and refers to the stimulation of phytoplankton or macro fit (Algae and plants) growth in aquatic environment [22]. To date, in all modeling studies, only effects of mari culture originated nutrient waste on phytoplankton growth were investigated. Especially, phytoplankton growth in summer months controlled by nutritious elements (Nitrogen, phosphate etc.) were modeled. Factors of this kind of model may lineup as; determination of bottom structure, water column affected from waste and dilution rate, calculation of nutrient level and determination the relationship between phytoplankton growth and nutrients [22].

In Baltic Sea, relations between sea water sensitivity, (depends on the interaction between open water and coastal water) nutrient concentration, phytoplankton biomass and secchi depth compared by using multiple regressions techniques [23]. Depending on the sensitivity of the coastal areas, while phytoplankton biomass increases, secchi depth decreases. But since the water in shallow areas is under influence of wave-induced re suspension, secchi depth method is unreliable. Therefore, the most accurate method for determination of the phytoplankton biomass is the measurement of chlorophyll content. In another study, direct and indirect effect of eutrophication was investigated between 2001-2011 in Baltic Sea by using nutrient concentration, chlorophyll-a, and secchi depth indicators [24]. Study revealed that entire open Baltic Sea was affected by eutrophication between 2007 and 2011.

Chemical Pollution

A variety of chemicals used for disinfection and disease outbreak treatment in aquaculture [25]. Environmental impacts of chemical compounds depend on toxic effect of the compound, pathogens sensitivity to antibiotics, therapeutics and active time zone of the chemical in the environment. Most of the chemicals were adapted from the poultry and cattle husbandry and their possible effect on the marine environment isn't fully investigated.

Chemicals in use in mariculture can be classified as disinfectants, anti foulings and veterinary medicines. Main veterinary medicines are antibiotics, anesthetics and pesticides. Veterinary medicines are widely used in fish farms but it is not so common in crustacean and bivalve cultures. Most of the chemical treatment scenarios in Mariculture result in direct release of chemicals into water column and sediment. For the cage systems Atlantic salmon, rainbow trout, sea bass and sea bream are the most common fish species and most of the chemicals used for which cause pollution. Among numerous definitions of marine pollution the most common one is GESAMP's (Joint Group of Experts on the Scientific Aspects of Marine Pollution), which states that marine pollution is "the damage to living creatures in the environment, the hindrance to marine activities including fishery, the inability to use sea water and the reduction of amenities as a result of changes in environmental conditions due to deleterious effects induced by various waste materials and/or energy left to the marine environment (including estuaries) by people directly or indirectly» [22].

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When a disease emerges, farmers try to control the outbreak by using antibiotics. As a result of that, antibiotic makes its way through water by uneaten antibiotic treated feed or feaces. In order to control external parasites such as sea lice, different kinds of pesticides are used. Pesticides could be given to fish via feed or by bath. Pesticides such as ivermectin have high toxic effects on marine organisms. Bio fouling is one of the major problems in cage farming. Proliferation of bio fouling organisms adds considerable weight to open water cage net which threats the stability of the structure and readily reduce water flow through which reduces water quality within the cage systems [26,27]. Anti-fouling paints and coatings are used in order to slow growth of such organisms on net to maintain water flow through to systems. Active matter of such paints is generally copper. Degradation of the paint in time causes copper to penetrate water, which poses toxic effects on marine creatures. Another toxic metal originated from aquaculture is zinc. Zinc sulfate (ZnSO4) is used as feed additive to prevent disease outbreaks.

In 2001, government of Canada surveyed surrounding waters of the salmon cultures and find out that copper and zinc concentrations are higher than legal limit in 48% of the cage systems [28]. 0.15 mg/L copper is lethal for shrimps [29]. For urchin 1.4-11.4 ppm copper and 0.327 ppm zinc is lethal [30]. For amphipoda, 0.8ppm zinc causes 23% percent mortality [31]. When both copper and zinc present together in the environment their synergistic toxic effect becomes greater than the sum of their individual effects [32]. Metals generally have sublethal effect on aquatic organisms. Copper and zinc toxicity inhibit chemo receptors and damage digestion system. Acute exposure to copper inhibits both uptake and excretion of ammonia in rainbow trout [33]. It also makes the organism an easy prey for predators by disrupting their nervous system [34].

Antibiotics

Chemicals have wide range of usage in fish health management [35]. Report prepared by GESAP and WHO (World Health Organization) mention chemical usage pose a threat to human health. Despite the reduction in antibiotic and organophosphate use in aquaculture [36], synthetic parathyroid, colorant, preservatives, anti-parasitic and other chemicals that cause marine pollution are still in use [37]. Use of chemicals not only affects marine environment but also pose a risk to the farmers as well [22]. Indiscriminate use of antibiotics for fish health management is one of the major problems in aquaculture. Unmetobolized antibiotics are often passed to the aquatic environment by uneaten antibiotic treated feed and by faeces. In both water column and sediment, different classes of antibiotics have different half-life. oxytetracycline which is one of the most common antibiotics can be effective up to 30 days in both water and sediment. In fact, oxytetracycline is still measurable on sediment after several months without being biologically active. In cage systems, chemicals in uneaten antibiotic treated feed and faeces scatter to the surrounding environment by waves and current. Wild fish and filter feeders such as mussels and oysters consume antibiotic treated feed or it passes to sediment.

Bioactive substances such as antibiotics and pesticides are generally used for controlling disease and parasite in aquaculture [38]. Success or failure in aquaculture depends on the correct usage of chemicals, usage duration and usage conditions against infectious diseases and parasites. Effects of bioactive matters on environment can be align as;

I. Substances with inhibitory effect last long in animal tissue.

II. Release of substances with inhibitory effect to the aquatic environment.

III. Development and transmission of the antibiotic resistance gene in microorganisms.

Substances with inhibitory affects last long in animal tissue

Bioactive substances last long in living cells more than expected. For instance, trimethoprim in used in disease treatment, can be detectible in a mussel after 77 days [39]. In order to totally eliminate oxytetracycline and other sulfonamide based antibiotics from trout body above 10°C, at least 60 days should pass after the last usage [40]. Frequent use of antibiotics to prevent or treat bacterial fish diseases, may result environmental bacteria and intestinal bacteria to gain antibiotic resistance [41,42]. Antibiotics make their way to muscles through digestion system. The emergence of antibiotic resistance bacteria as a result of massive use of chloramphenicol was determined in trout farms, in Italy [43]. Antibiotic taken into body through digestion emits from intestines and passes to vascular system, from where is passes to muscles. Every antibiotic has a different withdrawal period. Since the muscle tissue of the fish carries antibiotics for a specific period of time after the treatment, fish should be kept in water for a while depending on the water temperature in order to eliminate the antibiotic residue from the tissues before putting fish on market. For instance, fish treated with oxytetracycline shouldn't be offered for sale at least for 20 days [44]. Especially in summer months along with temperature rise, mortalities in market size fish force farmers to use antibiotics. But farmers generally don't heed withdrawal time procedures. These antibiotic containing fish offered to market before the withdrawal period pass to the consumer causing bacteria in human body gain antibiotic resistance.

Releasement of substances with inhibitory effect to the aquatic environment.

Frequent use of the inhibitor substances in aquaculture and replacement of potential bioactive substances to the aquatic environment is frightening. Only 20-30 percent of the antibiotic treated feed is eaten by fish, whereas 70-80 % of it passes directly

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to the aquatic environment [45,46]. Oxytetracycline decay fast in marine environment but most of them bind to the tiny particles and accumulate on the sediment [47,48]. Accumulated oxytetracycline could still exist at enough concentration to exhibit activity even after 12 weeks [45]. Sediment that contains antibiotics effect the marine fauna negatively. For example, oxytetracycline residue was detected in blue mussel (*Mytillus edulis*) 80 meters away from cages where the antibiotic was used [49].

While the antibiotics on the sediment surface lose its effect easily, antibiotics like oxytetracycline, oxolinic acid, sarafloxacin and flumequin within the sediment can be effective for up to 180 days. Antibiotics on the sediment become ineffective so easily not because of the degradation, but due to the spread. Antibiotics like sulfadiazine and trimethoprim have relatively less half-life within the sediment which is approximately 90 days. In the other hand, half-life of the florphenicol in sediment was calculated as 4.5 days [50]. Various natural and synthetic chemicals, such as dichlorvos, malachite green and derris rootare used in large amounts in mari culture worldwide. We might give an example in order to reveal total amount of chemical usage; in 1989, Norwegian farmers used 3488 kg of dichlorvos in order to control sea lice (*Lepeophtheirus salmonis*) outbreak. Disclorvos and most of the used chemicals cause serious impacts on environment and they are need to be used wisely [51].

Development and transmission of the antibiotic resistance gene in microorganisms

Antibiotics are widely used for controlling and treatment of both human and fish diseases [52]. The amount of antibiotic use in aquaculture and in human health management is nearly same [53]. Thus, frequent usage of antibiotic cause development of antibiotic resistance gene in both human and animal pathogens and treatment of the bacterial diseases with antibiotics is getting harder and harder each day. Antibiotics are not only using as disease controller but also as growth and feed conversion promoters. [54,55]. In 2000, Denmark banned all non-therapeutic uses of antibiotics. In 2006, EU banned the use of antibiotics as growth promoter. Antibiotics used at low doses increase the development of antibiotic resistance genes in human pathogens and opportunist environmental bacteria [56] and ease the path of gaining multiple antibiotic resistance of bacteria [57-59]. Plasmids (extra chromosomal elements) located in plasma of the bacteria carry resistance genes. Development of the antibiotic resistance gene arises from the changes in plasmid DNA and chromosomal DNA mutation.

Resistance plasmids (resistance, R) can be transferred between bacteria spp. Plasmids are transferred between bacteria in two different ways; Transduction and conjugation. Most of the bacteria species including pathogenic bacteria have plasmids [38]. Transfer of R Plasmids from resistant bacteria to sensitive bacteria cause different bacteria species develop antibiotic resistance gene [60,61]. Being transferable and independently replicateable plasmids are the main factor of wide spread of the resistance genes. Since the excessive use of antibiotics in aquaculture could promote development of antibiotic resistance genes in pathogens [13,62], most of the antibiotic treatments are inefficient. Roughly 16 different antibiotics are commonly in use in aquaculture. [63] Ampicillin, florphenicol, erythromycin neomycin, oxytetracycline and tetracycline are among them. Some of the bacteria gain resistance against these antibiotics inevitably [64]. In 1981, resistance of *Aeromonas salmonicida* the etiological agent of the furunculusis disease to oxitetracycline and was 4% [65], resistance ratio reached 50% in 1990 [66]. Presence of antibiotic resistance gene in the environment reduces effectiveness of the antibiotic [42]. Usage of the antibiotics as a growth promoter, leads development of antibiotic resistance in human pathogen bacteria and increases the risk of antibiotic resistance gene transfer [67]. Transfer of the R Plasmids from aquatic microorganisms to human pathogens [68] poses a potential threat to human health [69].

Impacts of Mariculture on Sediment

To date, the importance given to investigation of impacts of pesticides, antibiotics and synthetic paints on human health has not been given to the studies related to investigate the impacts of these chemicals on marine environment. After feeding fish in a cage, uneaten feed and faeces accumulate on the sea floor. Excessive accumulation of organic substances on sea floor leads excessive proliferation of microorganisms which causes reduction in oxygen level required for other benthic organisms (crustacean, fish etc.).

Disease outbreaks occur rarely in wild fish populations since they are scattered all over the oceans and seas, whereas high population density in aquaculture systems often leads to disease outbreaks. Accumulation of uneaten feed, faeces, chemicals and antibiotics causes formation of anoxic zone on sea floor thus, most of the benthic organisms that live near to the salmon farms dissappeared, only some of the resistant bacteria proliferated [70]. In many countries, formation of antibiotic resistance in poultry, cattle and aquaculture borne bacteria threats human health. Antibiotics used against bacterial fish disease outbreaks are generally excreted to the water column [14]. These antibiotics accumulate on sediment or animals living in surrounding environment [71]. Scientists detected 9 different antibiotic compounds in different stream sediments. The concentration of the antibiotics ranged from 1.01μg/kg (sulfachloropyridazine) to 485μg/kg (sulfamethoxazole) [19]. Under suitable environmental conditions, antibiotic residues on sediment effect microbial communities and only resistant individuals can survive.

Impacts of the Pollution on Microbial Communities

In marine ecosystem, heterotrophic bacteria play an important role in biodegradation [72]. Bacteria are very

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sensitive to the environmental changes [73] thus they can be affected directly from anthropogenic nutrient input [74]. Studies clearly show that, the pollutions caused by aquaculture activities are changing structure and activity of benthic communities [75]. Chemical medicines are one of the most influential factors on bacteria communities. Direct release of antibiotic treated feed and medicines to the marine environment cause selective pressure on bacteria populations, fish pathogens and leads to development of antibiotic resistance genes [76,77].

Most commonly and widely used antibiotics groups in aquaculture are oxytetracycline, florphenicol and sulfonamide. Oxytetracycline taken by feed cannot be metabolized 100% by digestion. Some active quantity is excreted after metabolism and finds their way to water by faeces [22]. Antibiotics have a significant impact on the microbial populations that live on sediment. Biomass of the bacteria and balance of different species of bacteria is changing by antibiotic use. Bacteria living in sediment are responsible for nitrogen, phosphate and sulfate cycles and create the first step of food chain. Antibiotic contamination reduces the formation of sulfate and nitrate. Effects of this outcome on microbial communities are not clear. Another issue which needs to be known is, which compounds antibiotics degrade to after metabolization and what are the potential harmful effects of these chemicals. For instance, when florphenicol is taken by salmon, it transforms into florphenicol amine in fish body. To date, impacts of the metabolites substances on marine organisms have not been fully studied. The most studied topics are related with the development of the antibiotic resistance gene due to the accumulation of antibiotic residues in surrounding areas of the aquaculture facilities.

Pollution Originated from Construction/Building Materials.

Some of the construction materials expose heavy metals, plastic substances and by-products to the marine environment. Presence of these substances is generally unknown to farmers but we become aware of them day by day. The usages of preservatives, which are believed to be harmless, are gradually increasing. Plastic substances contain fatty acid salts, chromates, cadmium sulfide, antioxidants, UV conservatives, organophosphate, fungusites and disinfectants. Most of these composites are toxic for aquatic life but due to their low solubility and slower decay rate, their toxicity decreases.

Impact of toxic substances that leaks from construction materials of aquaculture facilities on environment are not fully understood. Docks, bottom of the vessels and nets painted with anti foulings contain effective and inexpensive chemical called tributyltin which is active substance of most of the antifouling [78]. Water pollution caused by tributyltin inhibits shell formation of oysters [79], kills mussel larvae, causes imposex, and endocrine disruption, in molluscs and also contaminates sediment, sea water, fish and other bivalve spp. Therefore, usage of substances that contains tributyltin is a big problem in some of the countries. Fortunately, worldwide use of thetributyltin as a antifouling agent has been banned by The International Maritime Organization (IMO) in 2003, new antifouling agents have been introduced to the market as a replacement.

Indirect Effect of Pollution on Human Health

Bacteria can adapt to the changing environment with presence of extra chromosomal elements (Plasmid) and horizontal gene transfers of the plasmids. Adaptation could be gained by realignment, duplication, by changing the copy number of the plasmids or by the lateral and horizontal movements of the plasmids between bacteria populations. Plasmids affect life functions of the host since the plasmids have vast range of bacteria host in nature and ability to multiply within the host. Resistance genes that developed on plasmids cause changes in genetic character of the bacteria and can be transferred both vertically (transfer of the genes between organism in a manner of reproduction) and horizontally (transfer of the genes between organism in a manner other than reproduction). In natural microbial communities genetic adaptations arise from presence of a plasmid via horizontal gene transfer. Transfer ratio of the plasmids in polluted environment is 2 to 10 times more than thatin unpolluted environment. Plasmids play an important role in development and spread of the antibiotic resistance genes [80-87].

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