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Review Article

Ecological Impact of Pesticides Principally Organochlorine Insecticide Endosulfan: A Review

Neelambari Vivekanandhan and Annadurai Duraisamy

CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Portonovo, Cuddalore district, Tamilnadu, India.

Corresponding author: nilbr23@gmail.com

Abstract:

The intensive use of pesticides resulted in dispersal and persistence of pollutants throughout the global environment. Bioremediation of pesticides in contaminated soil and water environments has become one of the most sensational issues due to their deleterious effect on public health and environment. In natural habitat, certain microbes are capable of metabolizing those persistent compounds or detoxify them which could be employed for bioremediation. Hence the direct use of such microorganisms capable of degrading xenobiotics is also becoming a popular approach to safeguard the environment. The review summaries the impacts and fate of the pesticides, especially endosulfan in polluted ecosystem.

Keywords: endosulfan, microbial degradation, pesticides

1. Introduction:

The wide-ranging use of pesticides had been reported to contaminate terrestrial and aquatic environments worldwide. FAO has defined the term of pesticide, "As any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies." Subclasses of pesticides include herbicides, insecticides, fungicides, rodenticides, pediculocides and biocides. Many pesticides can be grouped into chemical families. Pests can develop resistance to the indiscriminate use of pesticide, necessitating a new pesticide. Alternatively an increased dose of the pesticide can be used to counteract the resistance, which will worsen environment further. Prominent insecticide families include organochlorines, organophosphates and

carbamates. However, each pesticide substance has a particular behavior in the environment. Though it is known that, environmental persistence depends upon ambient conditions such as light, humidity, acidity and microbial activity, all of which can contribute to the breakdown of a compound. In many situations, the exact role of these factors and their interactions are not known. The effects of these substances on non target organisms, their accumulation in biota or their transfer to the higher trophic levels of the food-chain are discussed. The study detailed the impacts and fate of pesticides in particular, endosulfan in natural environment.

2. History of Pesticides:

Even before 2000 BC, humans have utilized pesticides to protect their crops. The earlier history of pesticides use is discussed in table 1. Muller and Korte, (1975) discovered DDT as an effective insecticide. Organochlorines such as DDT were dominant, but they were replaced in U.S. by organophosphates and carbamates. Since, then pyrethrin compounds have become the dominant insecticides. The first generation of pesticides introduced over 40 years ago includes the Organochlorine compounds (OC) and

Organophosporous (OP). Later they become unacceptably persistent in the environment, although at the time of their introduction, they seemed to be promising candidates.

In 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread (Daly *et al.*, 1998). Pesticide use has increased 50-folds and since 1950 and 2.3 million tons (2.5 million short tons) of industrial pesticides are now used every year (Miller, 2002). Seventy-five percent of all pesticides in the world are used in developed countries, but use in developing countries is in an increasing trend (Miller, 2004).

3. Impact of organochlorine pesticide:

Chlorinated pesticides viz., BHC, DDT, endosulfan, heptachlor, aldrin etc. have been used extensively for protection of crops and prevention of vector borne diseases. These chlorinated pesticides persist in the environment for very long periods, undergo bioaccumulation and biomagnifications and therefore impart toxicity to non-target organisms including human beings. Accordingly, manufacture and the use of these chlorinated pesticides has either been banned or severely restricted in most of the developed and developing countries.

Pesticide influences an unfortunate impact in the environment. In 1960s, it was discovered that DDT was interfering with the reproduction of many fisheating birds and cause a serious threat to biodiversity. Rachel Carson has written about this biological magnification in his best-selling book Silent Spring. The agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants (POPs), but it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes (Lobe, 2006). Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species in air, water and soil (Miller, 2004). Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to the soil contamination.

The Bhopal disaster occurred when a pesticide plant released 40 tons of methyl isocyanate (MIC) gas, a chemical intermediate in the synthesis of some carbonate pesticides. The disaster immediately killed nearly 2,000 people and ultimately caused at least 15,000 deaths.

The use of pesticides for the control of crop pests and weeds has increased. In addition to this, discharges from the pesticide manufacturing plants, accidental spills and natural processes as dilution, surface run off and leaching are the cause of the occurrence of xenobiotic compounds in surface waters. The effects of pesticides on aquatic environments are also due to their degradation products, which can be more toxic than the original substances (Thurman et al., 1992). There is evidence that the OP compounds are sufficiently persistent to reach the marine environment at concentrations high enough to affect aquatic fauna and flora. They are toxic to invertebrates and vertebrate aquatic animals at very low concentrations (ppb or ppm level). Shrimp and fish are particularly sensitive. The presence of pesticides in costal environment is not entirely unexpected, since major agricultural areas are located in coastal plains and river valleys. However, occasionally massive fish and shrimp kills have been reported in estuarine and coastal lagoon ecosystems. Although the agents for this have not clearly identified, agrochemicals from adjacent agricultural fields have long been suspected as the lethal toxicants involved.

4. Biomagnifications of pesticides:

Even small concentrations of chemicals in the environment can find their way into organisms, accumulate in food chains leading to higher doses causing problems. Biomagnifications occurs when the pollutant is long-lived, mobile, soluble in fats and biologically active. If a pollutant is short-lived, chances for breaking down before it can become dangerous are more. If it is not mobile, it will stay in one place and is unlikely to be taken up by organisms. If a pollutant is soluble in water it will be excreted by the organism. Pollutants that dissolve in fats are however, retained for a long time in the organisms. Biological magnification often refers to the process whereby certain substances such as pesticides or heavy metals move up in food chain, work their way into rivers or lakes and are eaten by aquatic organisms such as fish, which in turn are eaten by large birds, animals or humans. The substances become concentrated in tissues or internal organs as they move up the food chain. Pesticide degradation is a process involving three phases (Hatzios, 1991 and Shimabukuro, 1985). Phase I process involves oxidation, reduction, or hydrolysis, which changes the parent compound more water soluble and less toxic degradation product. Oxygenation is the most frequent first step in the biotransformation of pesticides. Many of these reactions are mediated by oxidative enzymes viz. cytochromeP450s, peroxidases and polyphenol oxidases. The second phase involves conjugation of a pesticide or pesticide metabolites to a sugar, amino acid or glutathione, which increases the water solubility and reduces the toxicity compared to the parent compound. Generally, metabolites obtained from phase II have little or no toxicity, and may be stored in cellular organelles. Finally, the third phase involves conversion of phase II metabolites into secondary conjugates, which are generally nontoxic.

Insecticides such as organochlorine hydrocarbons could be separated into dichlorodiphenyl ethanes, cyclodiene compounds other related compounds. and Chlorinated pesticides persist in the environment for very long periods, undergo bioaccumulation and biomagnifications and therefore impart toxicity to non-target organisms including human beings. They operate by disrupting the sodium/potassium balance of the nerve fibers, forcing them to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulation. Accordingly, manufacture and the use of these chlorinated pesticides had either been banned or severely restricted in most of the developed and developing countries.

5. Endosulfan:

Endosulfan (6, 7,8,9,10,10 – hexachloro – 1, 5, 5a, 6, 9, 9a hexa hydro – 6, 9 – methano 2, 3, 4 benzo (e) dioxathiepen-3oxide) is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. It is formed by the reaction of thionylchloride with thiodan. Endosulfan is a derivative of hexachlorocyclopentadiene and is chemically similar to aldrin, chlordane and heptachlor. Specifically, it is produced by the Diels-Alder reaction of hexachlorocyclopentadiene with *cis*-butent-1, 4-diol and subsequent reaction of the adduct with thionyl chloride. Technical endosulfan is a 7:3 mixture of stereoisomers, designated α and β . α and β -endosulfans are conformational isomers arising from the pyramidal stereochemistry of sulfur. α -Endosulfan is the more thermodynamically stable of the two, thus β -endosulfan irreversibly converts to the α form, although the conversion is slow. These two isomers persist longer under more acidic conditions. The dissipation depends on a multitude of factors such volatilization, alkaline hydrolysis and photodecomposition, besides the presence of fertilizer, crop pattern, atmospheric temperature, rain and biotic conversions among others (Goebel *et al.*, 1982).

Endosulfan was introduced in early 1950s. In1954 Hoechst AG (now Bayer Crop Science) won USDA's approval of endosulfan in the US. The Australian Pesticides and Veterinary Medicines Authority, the responsible federal authority declared that shunning evidence of the dangers is available regarding the use of endosulfan. The chemical has been banned worldwide by the Stockholm Convention of Persistent Organic Pollutants due to its unacceptable neurological and reproductive risks to farm works and wildlife. Many International studies identify endosulfan as an endocrine disruptor which interferes with hormones and reproduction.

Endosulfan is subjected to long range atmospheric transport, i.e. it can travel long distances from where it is used. The endosulfan commonly contaminates air, water, plants and fish of national parks. Most of these parks are far from areas where endosulfan was used. Endosulfan had also been detected in dust from the Sahara Desert collected in the Caribbean after blown across the Atlantic Ocean (Ramnarine and Kristy, 2008).

6. Ecological impacts of Endosulfan:

Endosulfan is extremely toxic to fish and aquatic invertebrates (Sunderam *et al.*, 1992) and it has been implicated increasingly in gonadal toxicity (Sinha *et al.*, 1997), genotixicity (Chaudhuri *et al.*, 1999) and neurotixicity (Paul and Balasubramanian, 1997; Siddique *et al.*, 2003) while provoking chronic symptoms like testicular and prostate cancer (Saiyed *et al.*, 2003) breast cancer and sexual abnormality (Arnold *et al.*, 1996) in numerous mammalian species. Persistence of endosulfan in soil and water environments had been widely reported by different researchers under different conditions (Rao and Murty, 1980; Guerin and Kennedy, 1992 and Sethunathan *et al.*, 2002). Because of its abundant usage and potential transport, endosulfan contamination is frequently found in the environment at considerable distances from the point of its original applications (Man singh and Wilson, 1995 and Miles and Pfeuffer, 1997).

DeLorenzo et al., (2002) reported on the bioconcentration factors (BCF) for freshwater green (Pseudokirchnereilla subcapitatum) algae and freshwater water flea (Daphnia magna) as 2682 and 3278, respectively, with little evidence on bioaccumulation in phytoplankton and zooplankton. In contrast, endosulfan residues in farmed snakehead and carp fish (Channa striata and Catla catla), from a region in India with intensive agricultural activities, revealed higher bioaccumulation factors (BAFs) of log 4.5 (±0.45) (Amaraneni, 2002). Endosulfan is classified as a priority pollutant by international agencies (Keith and Telliard, 1979). High doses of endosulfan in humans primarily affect the central nervous system, resulting in respiratory depression, dry mouth, convulsions and possibly death due to major biotransformation products like Endosulfan sulfate and Endosulfan diol are formed. Endosulfan sulfate is more toxic and more persistent compared to the parent Endosulfan (Kaur et al., 1998).

7. Endosulfan toxicity:

In an assessment of Endosulfan toxicity to a range of freshwater organisms representing various tropic levels in farm ditches in the Lower Fraser Valley of BC, Canada. Wan et al. (2005) found that endosulfan to be the more toxic of the two isomers. However, more importantly, a combination of the isomers and endosulfan sulfate appeared to be more potent than any single endosulfan isomer. The study concluded that the higher concentration of endosulfan used in Lower Fraser Valley farms drained into salmon which often exceeded the 96-h LC₅₀ values of juvenile salmon. The concentration measured in ditch sediments were negatively affect the development and survival of benthic organisms.

For biotic (microbial) degradation, Guerin (1999) found extensive degradation of endosulfan (N 85%) by indigenously mixed microbial populations under anaerobic conditions, but did not find significant enhancement of degradation from enriched inoculate. These results demonstrate the biodegradation potential of α -endosulfan in low oxygen containing environments such as sediments

and this may have implications for interpreting historic accumulation trends of the α -isomer in freshwater sediments. Endosulfan sulfate is likely to represent the predominant residue of endosulfan over time (Guerin, 2001). Aquatic half-lives have been reported to be respectively 23-27 h and 22-27h for α -and the β -isomers depending on the initial nominal concentration (Jonson and Toledon, 1993). Pesticides and other xenobiotics used in the shrimp farm and agriculture are posing a risk to a wide flora and fauna. The biological effects of endosulfan have been shown recently to be more significant than thought previously. Endosulfan poisoning was found to stimulate the central nervous system. Apart from its toxic effects on human health and animals, it is considered extremely toxic to beneficial microbial communities as reported by Buff et al., 1992. A comparison of acute toxicity values indicated that endosulfan was more toxic to giant water prawn, Macrobranchium rosenbergii compared to Ametryne spp. as investigated by Lombardi et al., 2001. However the USEPA has classified endosulfan in category I as highly hazardous pesticide based on the results of another study in which the LD₅₀ value was calculated as 18 to 160 mg/kg for rats, 7.36 mg/kg for mice and 77 mg/kg for dogs. The acute toxicity of α -isomer is three times more than of the β -isomer against mammals and the neurotoxicity caused by insecticide has been mainly attributed to α -isomer investigated and reported by Sutherland et al., 2004. Magesh and Kumaraguru 2006 reported on acute toxicity of endosulfan to the milk fish available at the south east coast of India.

8. Endosulfan degradation:

Endosulfan is subjected to both biotic and abiotic degradation in the environment that may result in oxidation to the corresponding sulfate or hydrolysis in aquatic systems to endosulfan diol. In turn the diol may degrade further to endosulfan ether, endosulfan hydroxyether or endosulfan lactone (Walse et al., 2003).

9. Microbial Endosulfan Degradation:

In an international programme in 1984 on chemical safety held in WHO Geneva, reported on the environmental health criteria of endosulfan and according to it, it is broken down by photolysis, hydrolysis and biodegradation. Microbial degradation plays a major role in degradation of endosulfan contaminated sites in the environment (Shetty et al., 2000). Microbial degradation or microorganisms play an important role in the pesticide degradation in the natural environment. Microorganisms that are capable of degrading pesticides detoxify pesticides and influence the fate of pesticides in the environment. In general, microorganisms demonstrate considerable capacity for the metabolism of many pesticides. Although they are capable of catalyzing similar metabolic reactions as mammals and plants, they possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds. There are two major types of microbial degradation of organic chemicals. The first, termed catabolism, is a type of degradation in which the organic chemical or a portion there of is completely degraded (e.g. mineralized) and the energy or nutrient gained contributes to cell growth. The second, incidental metabolism or co-metabolism, involves the partial degradation of an organic chemical with no net benefit to the organism, the compound being merely caught up in some metabolic pathway during the normal metabolic activities of the microorganisms (Racke, 1993). Microbial communities define the magnitude and pathways of organic matter, nutrient and energy dynamics in aquatic systems. Lynch and Hobbie (1988) reported on the metabolic activities of indigenous microorganisms in the environment which cause extensive modifications in the structure and toxicological properties of the contaminants resulting in the complete conversion of xenobiotics into innocuous inorganic end products.

Martens (1976), reviewed on 16 fungi, 15 bacteria, and 3 actinomycetes which were capable of metabolizing more than 30% of the applied endosulfan. The major metabolites detected were endosulfate, formed by oxidation of the sulfite group, and endodiol, formed by hydrolysis of the ester bond. The majority of highly active fungi formed endosulfate as the major metabolite, whereas the majority of active bacteria formed endodiol. Kumar and Philip, 2006 isolated three novel bacterial species namely, Staphylococcus sp., Bacillus circulans - I, and Bacillus circulans - II, from contaminated soil of a pesticide manufacturing industry and assessed their potential for the degradation of aqueous endosulfan in aerobic and facultative anaerobic conditions. After four weeks of incubation using mineral salt medium, mixed bacterial culture was able to degrade $71.82 \pm 0.2\%$ and 76.04 ± 0.2% of endosulfan in aerobic and facultative anaerobic conditions respectively, with

an initial endosulfan concentration of 50mgl⁻¹. Among the three species, *Staphylococcus* sp. utilized more beta endosulfan compared to alpha endosulfan in facultative anaerobic system, whereas *Bacillus circulans* – I and *Bacillus circulans* – II utilized more alpha endosulfan compared to beta endosulfan in aerobic system.

Jayashree and Vasudevan (2007), investigated on the effect of Tween 80 added to the soil on the degradation of endosulfan by a Pseudomonas aeruginosa strain at different pH (7.0 and 8.5). The addition of synthetic surfactant Tween 80 enhanced the solubility and degradation of endosulfan. A significant degradation (94%) was observed in pH 8.5 in the Tween 80 added soil. The degradation of both the isomers were observed and accompanied with the formation of endodiol and endosulfan sulfate. Kwon et al., 2005 isolated a bacterium, Klebsiella oxytoca capable of degrading endosulfan sulfate as well as endosulfan using mineral salt medium containing 150 mg l⁻¹ of endosulfan or 173 mgl⁻¹ of endosulfan sulfate. The biomass was rapidly increased to an optical density of 1.9 at 550 nm in 4 days and the degradation constants for α - and β endosulfan, and endosulfan sulfate were respectively 0.3084, 0.2983 and 0..2465 per day. Balerao and Puranik (2007) investigated on the soil fungi in endosulfan degradation. Totally 16 fungal isolates were obtained by enrichment of soil samples that had been exposed to endosulfan earlier. On the basis of tolerance to endosulfan, Aspergillus niger was selected as a potential organism which could tolerate 400 mg ml⁻¹ of endosulfan. Complete disappearance of endosulfan was seen on 12 days of incubation. Evolution of carbon dioxide during endosulfan metabolism indicated the complete mineralization of endosulfan. Change in pH of culture broth to acidic range supported the biological transformation. Thin laver chromography (TLC) analyses revealed the formation of various intermediates of endosulfan metabolism including endosulfan diol, endosulfan sulfate and an unidentified metabolite.

Awasthi *et al.*, 2000 examined a bacterial co-culture consisting of two strains of *Bacillus* sp., isolated from a contaminated site by selective enrichment technique using endosulfan as the sole carbon source which degraded endosulfan within 15 days in a culture flask, with a simultaneous increase in the bacterial biomass. Sutherland *et al.*, 2002 isolated an endosulfan degrading bacterial strain designated as ESD from soil inoculums after repeated culture with

the insecticide as the sole source of sulfur. Enrichment of the sample was done for isolation of selective bacteria for effective degradation of endosulfan. Awasthi et al., 2003 studied the detoxification of α and β - isomers of endosulfan by a defined co-culture of two Bacillus strains. The degradation of both isomers was accompanied by the formation of endosulfan diol and endosulfan lactone. Accumulation of the endosulfan sulphate was not observed during the reaction with either of the isomers. Kwon et al., 2002 isolated bacteria that degraded endosulfan without formation of endosulfan sulfate from various soil samples using endosulfan as the sole carbon and energy source. Among the 40 isolated bacterial strains KE-1 identified as Klebsiella pneumonia showed superior endosulfan degrading activity. Analysis of culture pH, growth, free sulfate and endosulfan and its metabolites demonstrated that KE-1 biologically degraded 8.72µg of endosulfan per ml per day when it was incubated with 93.9 μ g ml⁻¹ of endosulfan for 10 days and the process was without the toxic formation of endosulfan sulfate.

Kumar et al., 2007 determined that two mixed bacterial cultures i.e., Stenotrophomonas maltophilia and Rhodococcus erythropolis were able to degrade respectively 73% and 81% of α and β endosulfan with endodiol intermediate, after two weeks of incubation. The toxicity studies of endosulfan before and after degradation were carried out using micronucleus assay on human polymorphonuclear cells, S. maltophilia showed better degradation efficiency compared to that of R. erythropolis. Li et al., 2009 isolated Achromobacter xylosoxidans CS5 capable of utilizing endosulfan as the sole carbon, sulfur and energy source from the activated sludge of Jiangsu, China. Analysis of culture pH, cells growth and residual endosulfan demonstrated that CS5 could degrade more than 24.8 mg/l α endosulfan and 10.5 mg/l β endosulfan after 8 days in aqueous medium, with the formation of endosulfan diol and endosulfan ether as the major metabolites. Cell-free extract of strain CS5 was able to metabolize endosulfan rapidly, and the degradative enzymes were constitutively expressed. Inoculation of strain CS5 was found to promote the removal of endosulfan in soil and also suggested that A. xylosoxidans CS5 might degrade endosulfan by a non-oxidative pathway. To assess the degradation of endosulfan the chloride content was estimated according to Bergman and Sanik (1957) method.

Kumar *et al.,* (2008) analyzed the endosulfan degradation rate using GCMS for quantitative and qualitative analysis of the compound and its residues.

10. Conclusion and further research:

The study implies the impact and biomagnifications of organochlorine pesticide in living habitat. Even though the synthetic pesticides cause serious effects, certain native organisms adapted to the pesticide pollutants and even degrade into non toxic end products. Microbial bioremediation plays a major role in eradicating the concern for endosulfan toxicity. Many bacteria and fungi were reported to degrade and detoxify the endosulfan residues in environment, thus acting a major role in successful bioremediation of pesticide pollution.

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