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Uptake and accumulation of endosulfan isomers and its metabolite endosulfan sulfate in naturally growing plants of contaminated area

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ABSTRACT

Endosulfan isomers (α+β) and its main metabolite endosulfan sulfate were analyzed in naturally growing vegetation of pesticide contaminated area in Ghaziabad, India. Seven species of dominating plants were collected at different locations within the contaminated area. Endosulfan residues from plant parts and soil were extracted and determined by a gas chromatograph equipped with 63Ni electron capture detector (GC-ECD). Endosulfan isomers and endosulfan sulfate were present in almost all soil and plant samples. The concentration of total endosulfan in plant and soil samples analyzed ranged from 14 to 343 ng g^{-1} and 13 to 938 ng g^{-1} respectively. Out of seven plant species studied, *Vetiveria zizanioides* (Khus Khus) and *Sphenoclea zeylamica* (Chikenspike) showed the highest and lowest accumulation respectively, with a significant difference at $p<0.01$ level. *Vetiveria zizanioides* and *Digitaria longiflora* (Crab grass) could accumulate considerable levels of endosulfan isomers (α+β) (343 and 163 ng g^{-1} respectively) and endosulfan sulfate (21 and 2 ng g^{-1}, respectively). The outcomes of the study reflect the value of test species in monitoring purposes and their potential for remediation of contaminated sites.

1. Introduction

Endosulfan is a broad spectrum cyclodiene organochlorine pesticide that persists in the environment, bioaccumulates through food chain and poses a risk of causing adverse effect to environment and human being. Technical grade endosulfan is a mixture of two sterioisomers α and β endosulfan (7:3) (Mersie et al., 2003). It is used extensively throughout the world for controlling different insect pest of crops such as cotton, fruits, vegetables and cereals (Lopez-Blanco et al., 2002; Kumar and Philip, 2006). Endosulfan is of environmental concern because it is extremely toxic to various organisms (Velasco-Santamaría et al., 2011; Trekels et al., 2013; Tao et al., 2013) and has been associated with mammalian gonadal toxicity, genotoxicity and neurotoxicity (Sunderam et al., 1992; Paul and Balasubramaniam, 1997; Silva and Beauvais, 2010; Lafuente and Pereiro, 2013). Because of their widespread use, the isomers of endosulfan have penetrated into almost all parts of ecosystems (US-EPA, 2007, 2009). Endosulfan is listed as a candidate for new persistent organic pollutants (POPs) under the Stockholm Convention due to its persistence, bioaccumulation potential, ability to travel long distance and toxicity (POPRC, 2009). It is a potent carcinogen and teratogen and classified by World Health Organization (WHO) as category II (moderately hazardous) pesticide (WHO, 1991). However the United States Environmental Protection Agency (USEPA) classifies it as a Category 1b (highly hazardous) pesticide. Research shows that these compound once present in an environment, redistribute into the different media by diverse process (adsorption, volatilization, microbial degradation, leaching), which cause contamination of the whole ecosystem (Antonious and Byers, 1997; Antonious et al., 1998; Si et al., 2006; Gouin and Wania, 2007; Jia et al., 2010). Almost 81 countries have banned the use of this persistent compound, but it is still used extremely in India, China and many other developing countries (Watts, 2011). Indiscriminate use of endosulfan combined with its persistence and conscious degradation has led to a worldwide environmental problem. Therefore, the determination of endosulfan isomers and its metabolite in flora is of major implication for assessing the risk of transfer to the trophic level and for the development of plant based remediation techniques that can be applied to endosulfan contaminated soils.

There is a need to develop economically and ecologically safe, remediation technologies. The literature shows that plant acts as a sink for environmental contaminants (Ho et al., 2013). Phytoremediation is an emerging technology that aims to provide a cheap and safe treatment to contaminated sites (Sun et al., 2011; Mitton

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et al., 2012; Becerra-Castro et al., 2013; Huang et al., 2013). Monitoring of plants which are growing in and around the contaminated sites will provide a general vision on the allocation of pollutants in plant species and their potential to uptake the pollutants (Barriada-Pereira et al., 2005; Abhilash et al., 2008; Agamuthu et al., 2010). Contaminant levels in vegetation samples can be used as indicators of environmental pollution, as plants can absorb compounds through their roots as well as aerial parts (Krauthacker et al., 2001; Meijer et al., 2003; Peralta-Videa et al., 2009). The pollutant accumulator plants can be employed for decontamination of polluted sites (Aslund et al., 2007). Very few studies have been undertaken to determine the plant uptake of organochlorine and other pesticides from the soil (Kipopoulou et al., 1999; Fismes et al., 2002) except under circumstances where soils were highly contaminated (Barriada-Pereira et al., 2005; Abhilash et al., 2008). Pollutant exposure and accumulation period are mainly responsible for the contaminant level in the plants (Krauthacker et al., 2001; Barriada-Pereira et al., 2005).

The present study aims to identify: (i) the contamination level of endosulfan in the soil near endosulfan handling unit; (ii) accumulation pattern of endosulfan in naturally growing plants near pesticide handling unit; and (iii) distribution of endosulfan isomers and its metabolite endosulfan sulfate in different plant parts.

2. Materials and methods

2.1. Sampling location

The plant and soil samples were collected from the campus and nearby areas of Flora Chemical and Fertilizers Limited, Ghaziabad, Uttar Pradesh, India. This area is known for the production of crops such as cereals, sugar cane, pulses, oil seeds etc. The study area is located between 28°41′10″ North latitude and 77°27′40″ East longitude. This pesticide handling unit has been established in 1996 and is associated with preparation of formulations of different pesticides. Seven species out of the most abundant growing plants in the area were collected from 10, 20 and 30 m away from dump site of the studied area (Table 1) and samples were washed, separated into different matrices, air-dried and stored at 4 °C for further analysis. Since it was monitoring study, random sampling was done.

To analyze the extent of endosulfan pollution in the soil we have also collected soil samples ($n=3$) from seven different points (1 m away from each plant) of the studied area up to a depth range of 0–15 cm. The upper layer and the whole particle organic matter were discarded. The bulk soil samples were grounded and passed through 2 mm sieve to remove stones and plant material, after which the samples were homogenized, dry ice stored to transport from field to lab.

2.2. Chemicals and standards

Individual standards of α endosulfan, β endosulfan and endosulfan sulfate were obtained from Supelco (Bellefonte, PA, USA). Ethyl acetate, n-hexane, acetone, dichloromethane gradient quality were purchased from Burdict & Jackson, Germany. Florisil (60–100 mesh size) of Sigma-Aldrich, USA. Na_2SO_4, NaCl and $MgSO_4$ of Merck Chemicals, India were purchased for extraction and clean up.

Table 1
Vegetation collected from contaminated area.

S. no.	Plants	Family	Matrices
1.	*Sonchus olerceous* Linn.	Asteraceae	Root, shoot, leaves
2.	*Digitaria longiflora*	Poeaceae	Root, shoot, leaves
3.	*Panicum palndosom* L.	Poeaceae	Root, shoot, leaves
4.	*Chloris virgata*	Poeaceae	Root, shoot, leaves
5.	*Sphenoclea zeylamica*	Sphenocleaceae	Root, shoot, leaves
6.	*Sacciolepis interrupta* (wild) staff	Poeaceae	Root, shoot, leaves
7.	*Vetiver zizanioides*	Poeaceae	Root, rhizome, leaves

2.3. Extraction and clean up

The samples were extracted by the EPA 8081A method with slight modifications and finally determined by GC-ECD. Leaf, stem and root samples were dried at 35 °C for 24 h, powdered, sieved (1–2 mm) and stored at 4 °C for posterior analysis. Powdered plant matrices (5 g) were gently ground in a pestle and mortar for 5 min and 1 g $MgSO_4$ and 0.5 g NaCl was added to this mixture and ground firmly for five more minutes. This mixture was used for soxhlet extraction for 8 h with a mixture of hexane dichloromethane solvent (50:50). Hexane (10 ml) was transferred to column filled with activated florisil (5 g) and anhydrous Na_2SO_4 (2 g). Soxhlet extract was transferred to this florisil and Na_2SO_4 column. A mixture of n-hexane dichloromethane solvent (50:50) was utilized for the elution in the column and repeated with another 20 ml of the same solvent mixture. The same method was applied for the extraction of endosulfan from soil (10 g) by using n-hexane and ethyl acetate solvent mixture. When necessary further purification was performed using a co-column (florisil + anhydrous Na_2SO_4). The resulting extract was concentrated up to dryness with a rotary evaporator. This extract was diluted with n-hexane (2 ml) and analyzed through GC-ECD.

2.4. Instrumentation and operating conditions

A Perkin Elmer (Norwalk, CT, USA) Clarus 500 GC equipped with a ^{63}Ni electron capture detector (ECD) was used for the detection and quantification of endosulfan isomers and its metabolites. A split/splitless injector was used in a splitless mode, applying injection volume of 1 μl. For separation a 35 percent diphenyl and 65 percent dimethyl polysiloxane capillary column (30 m × 0.32 mm ID × 0.5 μm) Elite 5 (Perkin Elmer, USA) was employed. The injection temperature was 250 °C and detector temperature was 300 °C. Nitrogen (N_2) of purity greater than 99.999 percent was used as a carrier gas at programmed flow of 1.0 ml/min. Oven temperature was kept at 170 °C (2 min), with an increase of 10 °C/min to 250 °C. The peaks of α endosulfan, β endosulfan and endosulfan sulfate were obtained at retention time of 12.51 min, 13.67 min and 14.62 min respectively (Fig. S1).

2.5. Accuracy and precision

Quantification of endosulfan isomers and its metabolite endosulfan sulfate was performed by using a standard curve prepared by injection of the known concentration of standard solutions of the mixture of the two endosulfan isomers and one metabolite endosulfan sulfate in n-hexane. In this study precision, linear dynamic range and both instrumental and method detection limits were evaluated for the method utilized by following analytical detection limit guidelines (ADLG, 1996).

Intra-day precision (repeatability) were measured on a single day using three replicates of spiked matrices under the same conditions (same analyst, apparatus, reagents and short interval of time) whereas inter-day precision was calculated during four consequent days also using three replicates of matrices spiked at same concentration.

Method detection limit (MDL) was calculated by multiplying sample standard deviation by student's *t*-value while limit of detection (LOD) was calculated by multiplying standard deviation by three (ADLG, 1996).

The accuracy of the method was evaluated by determining recoveries, using samples spiked at different fortification levels (0.01 and 1 ng g^{-1}) (Table 2). Linearity was assessed by diluting standard solutions in n-hexane at five consecutive concentrations (0.01, 0.05, 0.1, 0.5, and 1 ng ml^{-1}). The response of detector was linear in the studied range of 0.01–1 ng ml^{-1} with correlation coefficient of 0.9968–9986.

2.6. Statistical analysis

The data were subjected to analysis of variances (ANOVA) $p<0.01$ to by using SPSS (16.0) software for windows program, followed by post hoc Duncan Multiple Range Test (DMRT).

Table 2
Mean percentage recovery of endosulfan isomers and endosulfan sulfate in various plant matrices at different fortification levels.

Matrix	Mean percent recovery range		
	α Endosulfan	β Endosulfan	Endosulfan sulfate
Root ($n=5$)	89–92	88–90	84–88
Stem ($n=5$)	90–92	89–91	87–90
Leaves ($n=5$)	88–91	86–89	88–90
Soil ($n=5$)	85–89	87–90	86–88