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Assessment of diverse phthalate esters (PAEs) from irrigated agriculture soil under protected cultivation in IARI, New Delhi

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Abstract
Phthalate esters (PAEs) belong to a broad group of compounds which include Dialkyl or alkyl aryl esters of 1, 2-benzene, Dicarboxylic acid (phthalic acid). They widely used as plasticizers in polyvinyl chloride plastics. The goal of present investigation was to demonstrate the protocol for the estimation of PAEs from contaminated sites, also standardized the recovery process and estimate the presence of different PAEs mainly DEP and DEHP from contaminated sites. Standardized the protocol to estimation of PAEs. The recovery estimated on different concentration of DEP and DEHP. The recovery obtained of DEP and DEHP in range of 90.93±0.404 to95.3±0.211. Estimated the residual PAEs in agriculture soils in range of 109 ppm to 120 ppm.

Keywords: Phthalate esters (PAES), biodegradation, DEP, DEHP and HPLC etc.

Introduction
In recent years, the worldwide increase of urbanization and industrialization has led to the release of some complex and toxic organic compounds into the environment. The presence of the contaminants called “emerging” contaminants (ECs) such as phthalates has been reported in many countries.

Phthalate esters (PAEs) are a group of compounds, which include dialkyl or alkyl aryl esters of 1,2-benzendicarboxylic acid (Phthalic acid). Phthalate esters or phthalic acid esters (PAEs) have acquired huge attention from environmental regulatory bodies owing to its endocrine disrupting anti-androgenic activity. Results of recent research have led to defining several PAEs as “environmental hormones” because they interrupt biological hormones (Kambia *et al*., 2001) [7] to cause great impact on living creatures. The long-term accumulation of PAEs in humans may interfere with the secretion of hormones and the normal function of the nerve and immune systems, causing significant damage.

Among PAEs, dimethyl phthalate (DMP), dimethyl terephthalate (DMT), diethyl phthalate (DEP) and dibutyl phthalate (DBP) are mostly used for a variety of commercial purposes and have been listed as priority pollutants by agencies such as U.S. Environmental Protection Agency. Phthalate esters, also have been widely used to increase flexibility, extensibility, and workability of plastic polymers as plasticizer, or to help make perfume fixatives, lubricants, adhesives, weather stripping, and safety glass. Many consumer products contain specific members of this family of chemicals, including building materials, household furnishings, clothing, cosmetics, pharmaceuticals, nutritional supplements, medical devices, dentures, children's toys, glow sticks, modeling clay, food packaging, automobiles, lubricants, waxes, cleaning materials and insecticides. Phthalates are synthetic compounds used predominantly as additives in plastic to improve mechanical properties of the plastic resin, particularly flexibility. An absence of plasticizer renders a plastic brittle or inflexible.

Due to their wide use, PAEs are found in human residential and occupational environments in reasonable concentrations due to their application in both industrial processes and consumer products (Cai *et al.* 2007 and Che *et al*. 2011) [1, 3, 4]. Various plastic materials are widely used in agriculture which includes soil fumigation film, irrigation drip tape/tubing, nursery pots and silage bags. Since phthalates are not chemically bound to the plastics but remain present as a freely mobile and leachable phase, their migration from soft plastic during the production

phase or via leaching/volatilization from plastic products in due course of their use in the environment and after disposal (Li *et al*. 2010 and Cheng *et al*. 2016) [8, 5]. So it is expected that soil may contain a high concentration of these PAEs which may find their way into the food chain.

Numbers of investigators have demonstrated successful degradation of several PAEs by microbes under aerobic condition in soil, natural water and wastewater. Although there are number of reports on the biodegradation of individual PAEs, there is scanty literature available on degradation of mixture of PAEs. Industrial wastes frequently contain mixture of PAEs and therefore it is important to investigate the biodegradation of their mixtures rather than individual PAEs.

Materials and Methods

The laboratory experiments were conducted at ICAR-Indian Agricultural Research Institute, New Delhi in two divisions namely, at Division of Microbiology and Agricultural Chemicals. The locations stand at 28.08°N and 77.12°E, the height above mean sea level being 229 m (or 750 ft).

Phthalate esters affected soil samples were collected from three different sites at Centre for Cultivated Protection Technology (CPCT) IARI, New Delhi, India. A total 5 composite soil samples collected at 0-15 cm depth using a soil auger. Each composite sample was made from five sub samples which was collected along the zigzag paths (Zigzag sampling) to account for the randomness. The collected soil samples were properly labeled and stored in polythene bags and transported to the laboratory.

Residual Phthalates Esters in Soil Using HPLC

Hundred gm of soil sample was taken in 250 ml conical flask. Added 10 ml water followed by 50 ml acetone. Kept the flasks on shaker for half an hour and then filled it. Again, added 10 ml acetone in the flask and filtered once again. Evaporate acetone using rotary evaporimeter after complete extraction. Added 50 ml aqueous NaCl (20%) to the residue and using separating funnel partitioned the contents with 50 ml di chloro methane (DCM). The lower layer was collected and again partitioned with 30 ml DCM and same procedure was repeated with 10 ml DCM also and further DCM was evaporated using rotary evaporimetery. Finally, 2ml methanol was added in the remaining residue in rotary evaporimeter and all the contents were filtered using 0.22 micron filter. The sample was estimated using HPLC. (Wang *et al*. 2013, Feng *et al*. 2005) [11, 6].

HPLC Conditions

HPLC analysis was carried out on a Hewlett Packard instrument (series 1100) equipped with degasser, quaternary pump, diode array detector (DAD) connected with Rheodyne injection system (20 µl loop) and a computer (model vectra). The stationary phase consisted of Lichrospher on C – 18 packed stainless steel column.

1. Column - Lichrospher RP – 18 (250 mm x 4 mm id)
2. Mobile phase - Acetonitrile – Water (80:20) (Isocratic)
3. Flow rate - 1ml/min
4. Injection volume - 20 µl
5. Wavelength - 246 nm
6. Retention time (RT) - 2.45 – 5.83 min for PAEs

$$Residual PAEs = \frac{Peak area of extracted sample \times concentration of stand. \times vol. of extract}{Peak area of standard \times volume of sample}$$

Recovery of Phthalate Esters from Soil

Ten gram of soil sample was taken in 250 ml conical flask. The soil sample was fortified with PAEs @1 ppm and added 1 ml water. The extraction was initiated with acetone and 30 ml acetone was added to the sample, mixed thoroughly by shaking for half an hour on mechanical shaker. After the setting down of the soil, the supernatant was decanted on filter paper and collected in the conical flask. Again, the residue was treated with 15 ml acetone with15 minutes shaking followed by decantation and filtration. Same procedure was followed using 5 ml acetone with manual shaking of 5 minutes. The collected supernatant was treated with 15% aq. NaCl and poured in a separating funnel, followed by addition of 50 ml DCM. The separating funnel was used for partition and collected the lower layer for further use. Followed by same procedure was repeated using 30 ml followed by 10 ml DCM and lower layer was collected in each steps. The whole collected sample was taken to rotary evaporimeter where DCM was evaporated and the residue was further treated with 2 ml methanol and filtered through 0.22 micron filter. The final titrate was estimated using HPLC. (Srivastva *et al*. 2010, kambia *et al*. 2001, Zeng *et al*. 2001) [9, 7].

Results

The standard protocol has been optimized for the estimation of different phthalate esters from soil and in MS broth. Also estimated the recovery of different PAEs from soil and MS broth medium. The recovery has been for DEP and DEHP in soil is 92.83±0.312% and 90.73±0.242% respectively in 100 ppm fortification of these PAEs. In the MS broth the recovery estimated more than soil and found in DEP and DEHP is 95.33±0.196% and 91.6±0.311% respectively in 100 ppm fortification of these PAEs. (Table 1)

Table 1: Recovery of DEP and DEHP

Fortification (µg mL^{-1})	Recovery (%)			
	DEP (Broth)	DEP(Soil)	DEHP (Broth)	DEHP (Soil)
	Mean± SD			
10 ppm	94.8±0.242	91.6±0.209	90.93±0.404	93.4±0.312
100 ppm	95.33±0.196	91.83±0.312	91.6±0.311	93.73±0.242
300 ppm	95.3±0.211	91.96±0.231	91.33±0.319	93.76±0.311

According to USEPA, 6 mgL^{-1}DHEP is permissible in water and this was the maximum concentration admissible and the threshold limit value of DEHP, DEP, and DMP are 0.55, 0.45 and 5.0 mgL^{-1} respectively. Hence, keeping these standards in mind in the present study to optimize and form standard protocol for the estimation of residual phthalate esters mainly DEP and DEHP from soil and MS broth, an experiment was carried out where both soil and MS broth fortified with three different concentrations mainly 10 ppm, 100 ppm and 300 ppm DEP and DEHP respectively.

Results obtained in Table 1 revealed that when DEP concentration was increased in broth from 10 ppm to 300 ppm the recovery of same compound was found in range 94.8% to 95.3% respectively. It was further observed that an increase of DEP concentration from 100 ppm to 300 ppm showed no effect on recovery resulting 95.3% recovery at 100 ppm as well as 300 ppm.

In case of soil, there was an increase in recovery of DEP from 10 ppm to 300 ppm showing 91.6% to 91.96% recovery respectively. Similarly the other important compound DEHP was also studied for recovery in MS broth and soil. The DEHP recovery increased from 90.93% to 91.33% along with increase in DEHP concentration from 10 ppm to 300 ppm in MS broth and it also increased in soil showing 93.4% to 93.76% recovery. In sample DK1, DK 2 and DK 3 found PAEs in range of 106 ppm to 120 ppm.

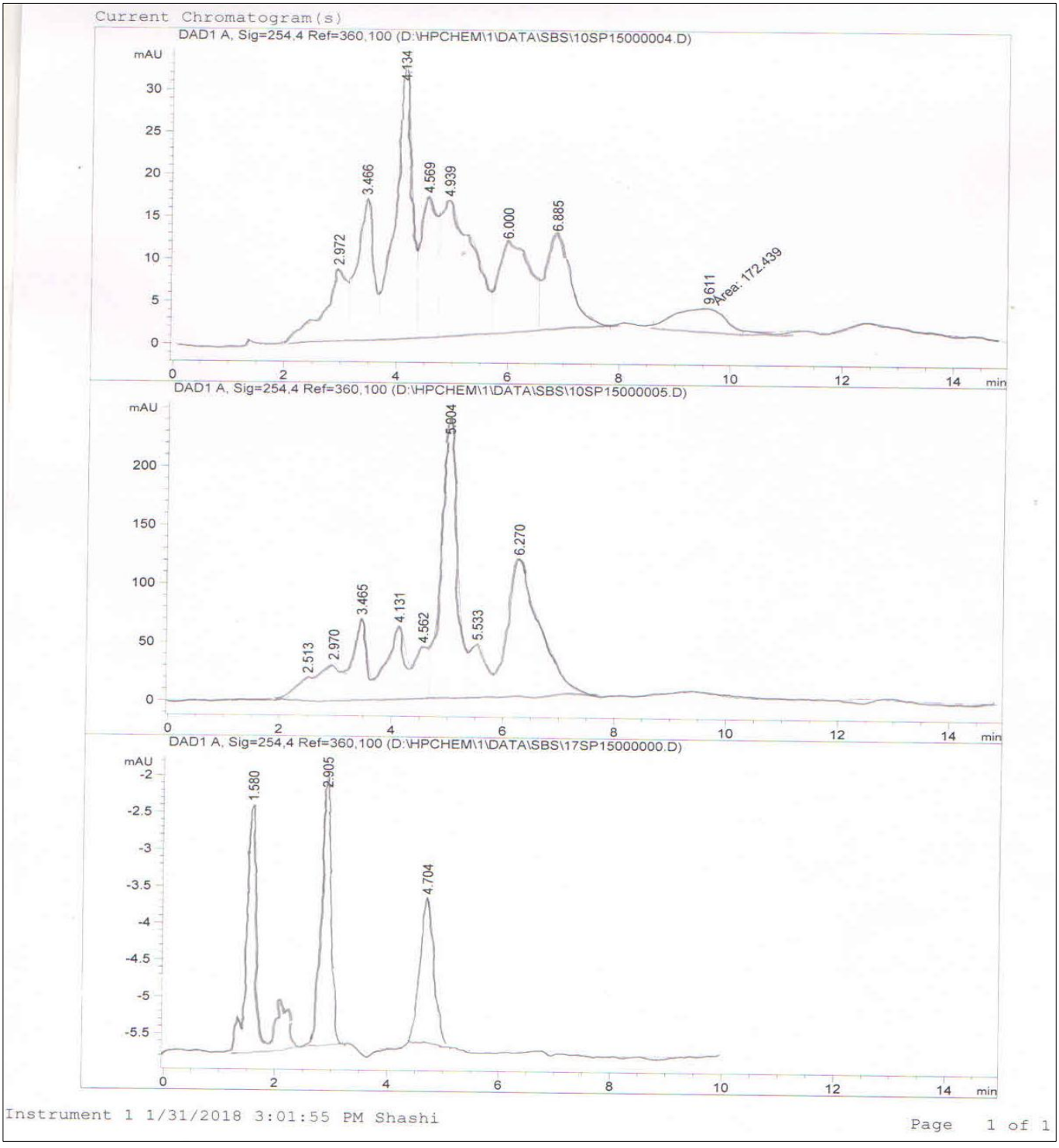

Fig 1: HPLC Chromatogram showing residual PAEs in contaminated sites

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