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QUANTIFICATION OF DEHP BY UHPLC IN SOIL OF MUNICIPAL WARDS OF PATNA, BIHAR

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ABSTRACT

Unrestrained manufacture and use of plastic has made the plasticizers ubiquitous in nature. Di-(2-Ehtylhexyl) Phthalate (DEHP) is one of the plasticizers used widely for imparting flexibility to the plastic, which is non-covalently bonded with the plastic matrix and leaches out due to change in pH and temperature. The main objective of the study is to evaluate the level of contamination of the soil of municipal wards of Patna with DEHP by using UHPLC. Soil samples were collected from 25 municipal wards at the depth of 30 and 50 cm of Patna urban area. The results of this study showed that DEHP is ubiquitously present in the top soil of all the selected municipal wards of Patna urban agglomeration. The concentration of DEHP ranged from 0.058 µg/g to 82.109 µg/g at the depth of 30 cm and 0.39 µg/g to 86.94 µg/g at the depth of 50 cm. The mean concentrations at the depth of 30 and 50 cm were observed to be 30.055 µg/g and 38.92 µg/g, respectively. This study also revealed that 18 out of 25 municipal wards were found to have higher than recommended permissible concentration of DEHP. Moreover, DEHP in the topsoil may pose a serious risk to the environmental and human health in this area. Therefore, extensive monitoring of other phthalates including DEHP must be taken up for addressing the potential threats to public health.

KEY WORDS : DEHP, Plasticizers,Phthalate, Patna, HPLC, Bihar

INTRODUCTION

Phthalate esters are one of the compounds used as plasticizer for providing flexibility to plastic products including PVC. Phthalate and its derivatives are commonly used in a wide range of plastic products, which further leach out into environment (Bertelsen *et al.,* 2013). These phthalate esters further percolate through the soil pores and tend to contaminate groundwater. Phthalates in soil and water make humans, animals and plants vulnerable to its exposure. Of all the members of the class of Phthalic Acid Esters (PAEs), Di-2-ethylhexyl phthalate (DEHP) is considered to be the most toxic. Di-(2-ethylhexyl) phthalate is commonly produced in industries at a large scale. DEHP is mostly used as a plasticizer in many plastic products, especially in medical applications, such as bags and tubes, nasogastric tubes, blood bags and infusion tubes, nutrition feeding bags, intravenous injections, umbilical artery catheters, peritoneal dialysis bags, and also in manufacturing a wide range of consumer products, such as soft plastic products, packed food and beverages, toys and infant products (Earls *et al.,* 2003). Due to unrestrained use of DEHP, it has become ubiquitous in the environment and hence, all humans, especially children are becoming susceptible to its exposure.

DEHP is not covalently bonded to the polymer matrix due to which it can be emitted during its manufacture, distribution and during and after its use. It is now evident that Food is the major route of exposure (UBA, 2012a) to DEHP to the general population, based on previous studies. Nevertheless, bottled water has been observed to be contaminated with DEHP, but still bottled water is not accounted to be a major route of exposure to DEHP (Diana and Dimitra, 2011; Fromme *et al*., 2007; Montuori *et al*., 2008; Schmid *et al.,* 2008). UBA (2012b) and Martine *et al*. (2013) have suggested that DEHP intake in human body through food is far greater than any other route but in general

population, the ingestion of DEHP can be a thousand times greater than water. Given the ubiquitous nature of DEHP in our environment, other ways for intake of DEHP is through environmental exposure (Bauer and Herrmann, 1997; EU, 2008; Guo *et al.,* 2011; Martine *et al.,* 2013; Staples *et al*., 1997; Wams, 1987). There are number of sources of exposure that include, but not limited to, in-door dust (Butte *et al.,* 2001; Bornehag *et al*., 2005), in-house air (Butte and Heinzow, 2002), soil (Cartwright *et al.,* 2000), and water (UBA, 2012a). A number of studies have demonstrated high levels of phthalate esters including DEHP in various soil types of agriculture (Niu *et al.,* 2014), vegetables (Li *et al*., 2016), urban (Cheng *et al.*, 2015; Wang *et al.,* 2018), electronics manufacturing area (Wu *et al.,* 2015), E-waste recycling waste site (Liu *et al.,* 2009), natural and reclaimed wetland (Wang *et al*., 2013) etc.

Moreover, UBA (2012b) has documented that routes other than food are minor in terms of intake of DEHP, and infants are the most vulnerable at a higher risk through indoor air and in-house dust and mouthing of plastic based toys. The crops and vegetables, grown at contaminated agricultural field (Li *et al*., 2018) or irrigated with contaminated water (Li *et al*., 2018), can readily uptake DEHP along with the water or nutrition from soil. UBA (2012a) and Wormuth *et al*., (2006) reported that 80% - 90% of the daily intake of DEHP in adult population was contributed via food. According to UBA (2012a), exposure of DEHP via bottled water is significantly lower than the lipid containing food items. The reported data cited above are, however, lower than the Tolerable Daily Intake (TDI) and Reference Dose (RD), which are based on the studies on non-cancerous effects used for standardization of various jurisdictions in different countries, such as 50 µg/kg of body weight (bw) in Europe (EFSA, 2005), 44 µg/kg bw in Canada (Health Canada, 1996) and 22 µg/kg bw in USA (USEPA, 1997).

Increasing number of reports raises the concerns over potent pernicious health effects related to ubiquitous exposures to plasticizers and the best hypothesis suggests that phthalates can act as endocrine disrupting agents (National Research Council, 1999). Specifically, multiple studies on humans as well as animals indicate that phthalates are capable of repressing key male hormones resulting into smaller anogenital distance and higher probability of undescended testes, leading to increased risk of carcinoma in testes and male infertility (Marsee *et al*., 2006; Swan *et al*., 2005).

Due to the deleterious effects of DEHP on environmental and human health, the current study has been undertaken in order to estimate the levels of DEHP in soil of 25 selected municipal wards of Patna urban agglomeration.

MATERIALS AND METHODS

Soil Sampling: Soil samples were collected from 25 municipal wards of Patna, which were randomly selected (Figure 1). Dumpsites from each Patna Municipal Wards were targeted for the collection of soil samples. The ground was dug up 30 and 50 cm for the collection of soil samples. A total of 50 soil samples from two different depths (30 and 50 cm) of 25 Patna Municipal Wards were collected for the estimation of DEHP concentration in soil. The samples were immediately transferred to the laboratory and stored in the deep freezer at - 20 °C for further analysis.

Geographic information system (GIS): Coordinates of each sampling sites were recorded by GPS system and used for generation of geographic information on the map of Patna, Bihar, India by using ArcGIS (version 10.2) (ESRI, 2014).

Microwave Assisted Extraction: Soil samples (5 g) were dried in hot air oven at 100-150 °C for 24 hours. The dried samples were ground with the help of mortar and pestle until fine powder like appearance was achieved. Further, 10 mL of acetonitrile was added to 2 grams of fine powdered samples in the ratio of 1:5 in 50 mL glass flasks. The flasks were closed air-tight and run in microwave for 30 mins. The samples were transferred to centrifuge tubes and centrifuged at 3000 rpm for 10-15 minutes. The supernatant was transferred to the test tubes and filtered through whattman filter paper. The final filtrates were collected in glass vials and stored in deep freezer at - 20 °C for further analysis.

UHPLC conditions: Ultra High Performance Liquid Chromatography (UHPLC) system DionexUltima 300 (ThermoFisher Scientific India Pvt Ltd), equipped with SD pump, vacuum degasser, manual injector, column compartment attached with Ultimate 3000 UV detector (VWD), was used for the quantification of DEHP in soil samples. Hypersil GOLD with 150 × 2.1 mm dimension was used as column for the separation. The temperature of the column was set at 30 °C. The maximum pressure was set at 620 bar. Flow rate of the solvents were

kept constant at 0.250 mL/min. The samples were again filtered by sterile syringe filter and injected manually with the volume of 20 µL. The gradient program in this study was designed as 80% B (0-7 min), followed by 100% B (7-11 min) and then reversed to the starting point, i.e., 80% B (11-20 min). Each elution run lasted for 20 minutes. All the data were acquired and recorded on Chromleon 7.2 software supplied by Thermo Fisher Scientific India Pvt Ltd along with UHPLC system.

RESULTS

Calibration: In order to quantify the concentration of DEHP in soil samples, UHPLC was standardized with DEHP. A varying concentrations of DEHP standard were prepared in acetonitrile as solvent by serial dilution. The concentrations of DEHP standard ranged from 1 to 100 µg/mL (1, 5, 10, 50, 100 µg/mL). The retention time of DEHP was detected to be 8.09 min (Figure 2). The mean ± Standard Deviation (SD) of recovery was observed to be 101.45 ± 3.67 % (Table 1).

Linearity Range and Accuracy: The linearity range of the DEHP standard ranged from 100 to 1 µg/ml (n=5). It was ensured that linearity range for DEHP was wide enough so that reliable data for the samples could be acquired. The standard Error (SE) of intercept was computed to be 0.0106, whereas standard deviation of the intercept was 0.0238. Limit of Detection (LOD) and Limit of Quantification (LOQ) were found as 0.895 µg/mL and 2.713 µg/mL respectively. The slope of the linear curve was 0.0877 and the intercept 0.0061 (Figure 3). Relative standard deviation was calculated as 1.806 %. Coefficient correlation (r) was found to be > 0.999 in all cases (Table 1).

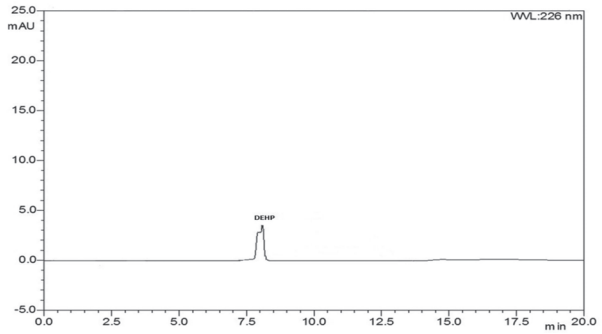

Fig. 2. Chromatogram of DEHP standard

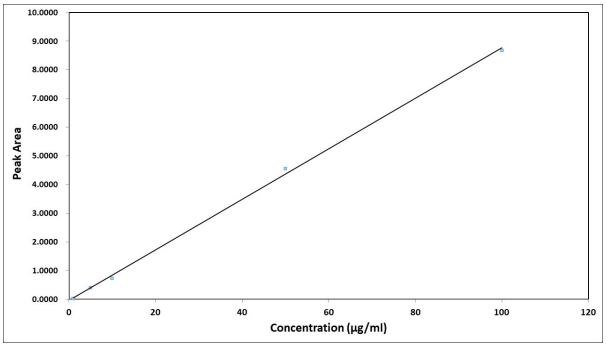

Fig. 3. Linear Curve of DEHP standard

Method Validation: In order to validate the method, the samples were added with standard of DEHP in

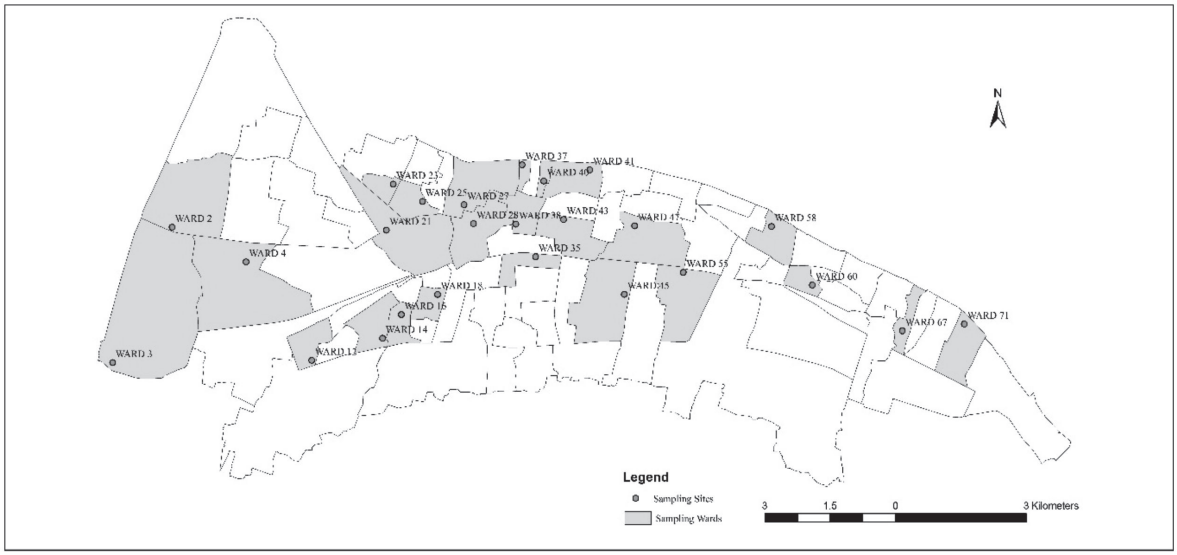

Fig. 1. Map of Patna urban agglomeration with wards demarcated by Patna Municipal Corporation indicating sampling sites.

the concentration of 0, 1 and 10 ng/mL and run on UHPLC. The experiments were repeated five times in order to achieve statistical significance. The mean recoveries of spiked samples of concentration of 1 ng/ml were 97.86%. On the other hand, mean recoveries of spiked samples of concentration of 10 ng/ml were 95.84 %. RSD of 1 ng/ml and 10 ng/ml spiked samples were 2.05 and 2.55 % respectively (Table 2).

Analysis

(a) Concentrations at depths: The developed method was validated for its applicability by determining the levels of DEHP in the soil samples of Patna municipal wards. The mean ± SD of DEHP level at the depth of 30 cm was found to be 30.05 ± 28.07 µg/g with 95 % of Confidence of Interval (CI) as 41.64 - 18.46 µg/g. Similarly, mean ± SD of DEHP concentration at the depth of 50 cm was calculated to be 38.97 ± 29.10 µg/g with 95% CI of 50.98 - 26.95 µg/g. However, the difference in the mean concentration of each DEHP at 30 and 50 cm depths was not found to be significant (p = 0.276) (Figure 4).

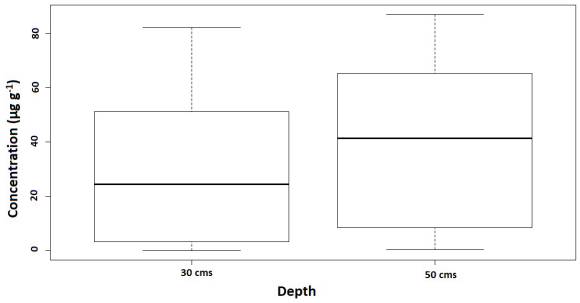

Fig. 4. Average concentrations (µg/g) (all wards) of DEHP at the depth of 30 cm and 50 cm.

(b) Concentrations in the municipal wards: The highest concentration of DEHP was observed to be 82.1 µg/g at the depth of 30 cm at ward number 16 (coordinates = 25.59138889, 85.11972222) followed by 78.32 µg/g at ward number 12 (coordinates = 25.57944444, 85.10305556), 72.40 µg/g at ward number 47 (coordinates = 25.60305556, 85.1525) and 64.3 µg/g at ward number 18 (coordinates = 25.5958333, 85.1319444) (Figure 5). Similarly, the highest concentration of DEHP was recorded to be 86.94 µg/g at the depth of 50 cm at ward number 21

A

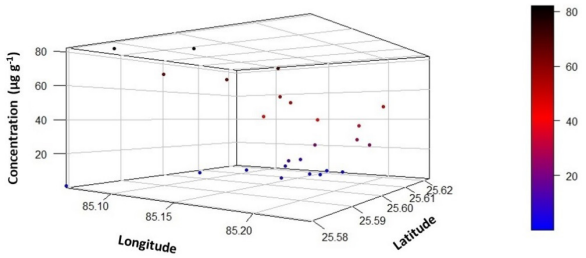

Fig. 5. Concentration of DEHP (µg/g) at the depth of 30 cm in 25 wards selected for the study

B

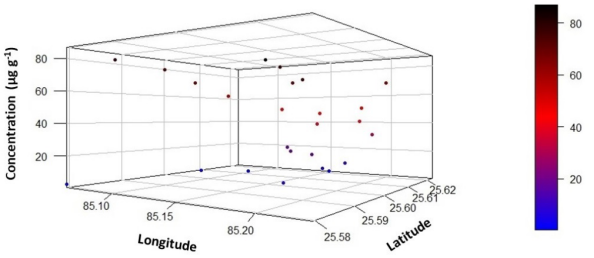

Fig. 6. Concentration of DEHP (µg/g) at the depth of 50 cm in 25 wards selected for the study.

Table 1. Analytical parameters of the method with linearity range, calibration equation, Coefficient correlation (r), Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Parameters	Values	Parameters	Values
Standard Size (n)	5	SE of Intercept	0.0106
Linearity Range (µg/mL)	100-1	SD of Intercept	0.0238
Retention time (min)	8.09	RSD (%)	1.806
Calibration Equation	y = 0.0877x - 0.061	r	0.999
LOD (µg/mL)	0.895	Accuracy	100.271 ± 1.806
LOQ (µg/mL)	2.713		

Table 2. Recoveries obtained in the determination of DEHP in spiked soil samples (n=5).

PAE	Spiked (ng/ml)	Mean Found (ng/ml)	Mean Recovery %	Mean RSD %
DEHP (n=5)	0	-	-	-
	1	0.97	97.86	2.05
	10	9.58	95.84	2.55

(coordinates = 25.61055556, 85.09) followed by 77.1 µg/g at ward number 47 (coordinates = 25.60305556, 85.1525), 76.2 µg/g at ward number 12 (25.57944444, 85.10305556) and 74.2 µg/g at ward number 24 (coordinates = 25.62, 85.13027778) (Figure 6).

DISCUSSION

The UHPLC was calibrated with standard of DEHP (>99% pure) and a linear curve was obtained with R^2 as high as 0.999 which suffices the fact that calibration is 99.98% linear and reliable (Figure 4.2). In a similar study by Saini (2018), UV-HPLC was calibrated with DEHP through C18 column and obtained the R^2 as high as 0.999 at 210 nm of VWD. The results were reported with high precision with relative standard deviation of ≤ 2.4% and relative error of ≤ 4% (Saini, 2018). The developed method was concluded to be rapid and highly reliable to estimate the trace levels of DEHP in water samples (Saini, 2018). The present study reflects that relative standard deviation was obtained as good as 1.806% with linearity range of 100 to 1 µg/mL at 8.09 min of retention time (n=5) where DEHP has been detected by UV at 226 nm (Table 1). Similarly, a study conducted to investigate the migration of DEHP from cap of beer bottle into the beverage, which included the method development for estimation DEHP in the beer sample (Li *et al*., 2012), the relative standard deviation was calculated to be 1.5 % and 0.6 % for 100 µg/mL and 200 µg/mL respectively at 11.35 min of retention time and 275 nm of VWD (Li *et al.,* 2012). Moreover, it must be noted that spiked samples produced with mean relative standard deviation as low as 2.05 % and 2.55 % for 1 and 10 ng/mL of spiking (Table 2).

The soil samples evaluated for the quantity of DEHP were found to be highly contaminated with DEHP in all the municipal wards. The mean DEHP quantified at the depths of 30 and 50 cm of the ground surface were 30.05 and 38.97 µg/g of soil of Patna municipal area (Figure 4). However, it was interesting to record that the difference between the levels of DEHP at the depth of 30 and 50 cm were found to be insignificant. The chief reason behind the observation of insignificant difference in the levels of DEHP at different depths of ground surface may be attributed to the fact that the soil of Patna urban area is Gangetic alluvium of Indo-Gangetic plain region, which is highly porous in nature allowing easy migration of DEHP through the soil depth. However, there were only two depth, i.e., 30 and 50 cm taken for the collection of sample. More number of depths with the last depth even deeper than 50 cm may yield difference and help understand the migration of DEHP through the soil of Patna urban area.

In a recent study by Tan *et al*. (2016), it was clearly demonstrated that PAEs in fine sand (53-250 µm diameter) tend to accumulate more amount of PAEs at the depths of 20 and 40 cm as compared to other soil particles (coarse sand (250-2000 µm), fine silt (20-53 µm) and clay (<2µm)) at the depths of 20 and 40 cm. However, the results also reflected that the quantity of PAEs in fine sand particles at the depth of 60, 80 and 100 cm was slightly less than other types of soil (Tan *et al.,* 2016). The study by Tan *et al*., (2016) corroborates the finding of this study that quantity of DEHP at 30 and 50 cm did not have significant difference as DEHP can efficiently migrate through fine sand, which is the major composition of Patna urban area.

The Patna urban area is majorly composed of coarse sand (250-2000 µm of diameter) at the bank of the Ganga river, fine sand (53-250 µm) in the radius of 3-7 km from the bank of river and silt (20-53 µm) or clay (<2 µm) after 7 km. Since fine sand is the major composition of surface soil of Patna, the findings are pertinent to the soil composition of Patna urban area. In another study, soil samples were collected at 20, 40 and 60 cm from Hamburg, Stuttgart, Berlin and Munich (Germany) and analysed for the presence of DMP, DBP and DEHP and the mean differences at different depths were also found to be insignificant (Schiedek *et al*., 1998), which suggests efficient migration of PAEs between 0-60 cm, which yields insignificant difference in the quantity.

The highest concentration of DEHP at the depth of 30 cm was observed in ward number 16, which is about 4-5 km south of the Ganga river which indicates the texture of the soil as fine sand (Figure 1). The soil texture of ward 16 is suitable for the accumulation of DEHP. Following ward number 16, the highest concentration of DEHP was found in ward numbers 12, 47 and 18 at the depth of 30 cm (Figure 5) which are also closer to the bank of the Ganga river (within radius of 4-5 km) (Figure 1) representing fine soil. Similarly, the highest concentration of DEHP at the depth of 50 cm was recorded in ward number 21, which is about 2-3 km from the bank of the Ganga that makes it suitable for the fine sand soil texture to deposit high amount of

DEHP (Figure 6). Following ward number 21, the highest concentration of DEHP at the depth of 50 cm was found in the ward numbers 47 and 24 (about 1.5 - 2.5 km from the bank of the Ganga (Figure 1), which represents even coarser particles of the soil making it suitablefor high migration of DEHP. The findings are supported by outcomes of the study reported by Tan *et al.* (2016), which documents highest PAEs accumulation in fine sand followed by coarse sand type soil at 20-60 cm. The outcomes of the present study also supports the fact that soil texture of Patna urban makes PAEs to migrate through soil to further contaminate groundwater.

Apart from presence of PAEs in water, air, sediments and soil, DEHP has also been reported in epoxidized soyabean oil, peanut butter, soft spreadable cheese and sauces (Pedersen *et al.,* 2008) and variety of vegetables (Fu and Du, 2011). Tran *et al*. (2015) reported a study, wherein, agricultural and non-agricultural soil samples of Paris, France were evaluated for the contamination with phthalates. The study reported few hundred of nanogram of DBP, DiBP, DnBP and Benzyl Butyl Phthalate (BBP) per gram of dry weight of sludge and few micrograms of DEHP, DiNP and DiDP per gram of dry weight of the sludge samples (Tran *et al.*, 2015). Interestingly, DEHP concentration at the junction of soil and sediment increased surprisingly 3-folds of the concentration recorded in the dry sludge samples (Tran *et al*., 2015). Moreover, half-life of DEHP at the depth of 0-20 cm was observed to be 64 days (Tran *et al*., 2015). The study concluded that urban soil was more contaminated with phthalates as compared to the agricultural land of Paris, France (Tran *et al*., 2015), which supports the findings of this study that Patna urban area is highly contaminated with DEHP. According to another report, the level of DEHP in soils of Sweden, Denmark, Germany and Norway ranged from 0.02 to 354 µg/g (JRC European Commission, 2008).

A burgeoning evidence of the mechanism of toxicity induced by PAEs suggests that phthalates, including DEHP act as endocrine disruptor (Grindler *et al.,* 2018). The consequences of action of phthalates as endocrine disruptor can be deleterious resulting in smaller anogenital distance, undescended testis, testicular cancer and male infertility (IARC 2000; Swan 2005; Mathers *et al*., 2009). Moreover, phthalate has been classified as a possible human carcinogen (Zeiger *et al*., 1982). Nevertheless, an extensive study on the soil contamination with PAEs and its impact on environmental and human health in Bihar state is required.

CONCLUSION

The present study includes the estimation of DEHP levels in soil samples of 25 Patna municipal wards. Plastic based products are used and dumped frequently, especially, in urban area. High level of DEHP in soil samples of 25 municipal wards has been observed. The average concentration of DEHP at the depth of 30 cm was found to be 30.05µg/g and that of 50 cm was 38.97µg/g. The soil texture of the city of Patna is composed mainly of alluvial soil, it makes it suitable for the deposition of DEHP. Moreover, fine soil allows smooth migration of the DEHP in soil making the underground water more vulnerable to the contamination with DEHP. The agriculture is the major activity in Indo-Gangetic plain, since the soil is highly fertile. The contaminated underground water used for irrigation or the contaminated agricultural land may lead to contaminated food. However, more data on underground contamination and agricultural land contamination with phthalates could help us understand the penetration of DEHP into our food chain. Furthermore, DEHP is considered to be endocrine disruptors and can manifest serious consequences on public health.

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Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

Bauer, M.J. and Herrmann, R. 1997. Estimation of the environmental contamination by phthalic acid esters leaching from household wastes. *Sci. Total Environ.* 208 (1-2) : 49-57. doi: 10.1016/S0048-9697(97)00272-6.

Bertelsen, R.J., Carlsen, K.C.L., Calafat, A.M., Hoppin, J.A., H°aland, G., Mowinckel, P., Carlsen, K. and Løvik, M. 2013. Urinary Biomarkers for Phthalates Associated with Asthma in Norwegian Children. *Environmental Health Perspectives*. 121 (2) : 251-

256.

Bornehag, C.G., Lundgren, B., Weschler, C.J., Sigsgaard, T., Hagerhed-Engman, L. and Sundell, J. 2005. Phthalates in indoor dust and their association with building characteristics. *Environmental Health Perspectives*. 113 (10) : 1399-1404. doi: 10.1289/ehp.7809.

Butte, W. and Heinzow, B. 2002. Pollutants in house dust as indicators of indoor contamination. *Rev. Environ. Contam. Toxicol*. 175 : 1-46.

Butte, W., Hofmann, W., Hostrup, O., Schmidt, A. and Walker, G. 2001. Endocrine disrupting chemicals in house dust: results of a representative monitoring (in German). *Gefahrst. Reinh. Luft*. 19-23.

Cartwright, C.D., Thompson, I.P. and Burns, R.G. 2000. Degradation and impact of phthalate plasticizers on soil microbial communities. *Environ. Toxicol. Chem*. 19 (5) : 1253-1261. doi: 10.1002/etc.5620190506.

Cheng, X., Ma, L., Xu, D., Cheng, H., Yang, G. and Luo, M. 2015. Mapping of phthalate esters in suburban surface and deep soils around a metropolis-Beijing, China. *Journal of Geochemical Exploration*. 155 : 56-61.

Diana, A. and Dimitra,V. 2011. Alkylphenols and phthalates in bottled waters. *J. Hazard Mater*. 185 (1) : 281-286. doi : 10.1016/j.jhazmat. 2011.09.031

Earls, A., Axford, I.P. and Braybrook, J.H. 2003. Gas chromatography-mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles. *Journal of Chromatography A*. 983 (1-2) : 237-246.

EFSA. 2005. European Food Safety Authority: Opinion of the Scientific Panel on food additives, favourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to bis (2 - ethylhexyl) phthalate (DEHP) for use in food contact materials-243. Question No. EFSA - Q - 2003-191. *The EFSA Journal*. 3 (9). doi: 10.2903/j.efsa.2005.243.

ESRI (Environmental Systems Research Institute). 2014. *ArcGIS Release 10.2*. Redlands, CA.

EU. 2008. European Commission, Institute for Health and Consumer Protection: EUR 23384 EN/2 Summary risk assessment report on bis(2-ethylhexyl) phthalate (DEHP). Vol. 80. Luxembourg.

Fromme, H., Gruber, L., Schlummer, M., Wolz, G., B¨ohmer, S., Angerer, J., Mayer, R., Liebl, B. and Bolte, G. 2007. Intake of phthalates and di(2 - ethylhexyl) adipate: results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. *Environ. Int.* 33 (8) : 1012-1020. doi : 10.1016/j.Envint. 2007.05.006

Fu, X.W. and Du, Q.Z. 2011. Uptake of di-(2-ethylhexyl) phthalate of vegetables from plastic film greenhouses. *Journal of Agricultural and Food Chemistry*. 59 : 11585-11588.

Grindler, N.M., Vanderlinden, L., Karthikraj, R., Kannan, K., Teal, S., Polotsky, A.J., Powell, T.L., Yang, I.V. and Jansson, T. 2018. Exposure to Phthalate, an Endocrine Disrupting Chemical, Alters the First Trimester Placental Methylome and Transcriptome in Women. *Sci. Rep.* 8 : 6086. doi:https://doi.org/10.1038/s41598-018-24505-w

Guo, Y., Wu, Q. and Kannan, K. 2011. Phthalate metabolites in urine from China, and implications for human exposures. *Environ. Int*. 37 (5) : 893-898. doi: 10.1016/j.envint.2011.03.005.

Health Canada. 1996. Health-based tolerable daily intakes Concentrations and tumorigenic doses/ Concentrations for priority substances. Ottawa.

IARC. 2000. Some industrial chemicals. IARC. *Monogr Eval Carcinog Risks Hum*. 77 : 1-529. PMID: 11236796.

JRC European Commission. 2008. Bis (2-Ethylhexyl) Phthalate (DEHP) - Summary Risk Assessment Report; Institute for Health and Consumer Protection Toxicology and Chemical Substance (TCS) European Chemicals Bureau. Italy, EUR 23384 EN/2.

Li, C., Chen, J., Wang, J., Han, P., Luan, Y., Ma, X. and Lu, A. 2016. Phthalate esters in soil, plastic film, and vegetable from greenhouse vegetable production bases in Beijing, China: Concentrations, sources, and risk assessment. *Science of The Total Environment*. 568 : 1037-1043.

Li, Y., Fei, F., Zhang, K., Chen, Q. and Li, Y. 2012. Migration analysis of DEHP from Inner Liner of Beer Bottle Caps by HPLC. *Procedia Environmental Sciences*. 12 : 17-21.

Li, Y., Huang, G., Gu, H., Huang, Q., Lou, C., Zhang, L. and Liu, H. 2018. Assessing the Risk of Phthalate Ester (PAE) Contamination in Soils and Crops Irrigated with Treated Sewage Effluent. *Water*. 10: 999. doi: 10.3390/w10080999.

Liu, W.L., Shen, C.F. and Zhang, Z. 2009. Distribution of Phthalate Esters in Soil of E-Waste Recycling Sites from Taizhou City in China. *Bull. Environ. Contam. Toxicol*. 82: 665-667. Doi: 10.1007/s00128-009-9699-3.

Marsee, K., Woodruf, T.J., Axelrad, D.A., Calafat, A.M. and Swan, S.H. 2006. The Undescended Testis: Diagnosis, Treatment and Long-Term Consequences. *Dtsch Arztebl. Int*. 106 (33) : 527-532.

Martine, B., Marie-Jeanne, T., Cendrine, D., Fabrice, A. and Marc, C. 2013. Assessment of adult human exposure to phthalate esters in the urban centre of Paris (France). *Bull. Environ. Contam. Tox*. 90 (1) : 91-96. doi : 1.1007/s00128-012-0859-5.

Mathers, M.J., Sperling, H., Rübben, H. and Roth, S. 2009. The undescended testis: diagnosis, treatment and long-term consequences. *Deutsches Arzteblatt International*. 106 (33) : 527-532.

Montuori, P., Jover, E., Morgantini, M., Bayona, J.M. and Triassi, M. 2008. Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. *Food Addit. Contam. A*. 25 (4) : 511-518. doi: 10.1080/02652030701551800.

National Research Council. 1999. Hormonally active agents in the environment. Washington, DC: National Research Council, National Academies Press.

Niu, L., Xu, Y., Xu, C., Yun, L.X. and Liu, W. 2014. Status of phthalate esters contamination in agricultural soils across China and associated health risks. *Environmental Pollution*. 195 : 16-23.

Pedersen, G.A., Jensen, L.K., Fankhauser, A., Biedermann, S., Petersen, J.H. and Fabech, B. 2008. Migration of epoxidized soybean oil (ESBO) and phthalates from twist closures into food and enforcement of the overall migration limit. *Food Additives and Contaminants*. 25 : 503-512.

Saini, S.S. 2018. Trace Analysis of di-(2-Ethylhexyl) Phthalate (DEHP) in Drinking Water Using MEPS and HPLC-UV. *Analytical Chemistry Letters*. 8 (1) : 9-24.

Schiedek, T., Grathwohl, P., Einsele, G. 1998. Occurrence and fate of plasticizers (phthalates) in soil: Laboratory and Field Studies. *Proceedings Sixth International FZK/TNO Conference on Contaminated Soil 'ConSoil 98',* Edinburgh, UK, Vol. 2, S. 881-882, Thomas Telford.

Schmid, P., Kohler, M., Meierhofer, R., Luzi, S. and Wegelin, M. 2008 Does the reuse of PET bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? *Water Res.* 42 (20) : 5054-5060. doi: 10.1016/j.watres.2008.09.025

Staples, C.A., Peterson, D.R., Parkerton, T.F. and Adams, W.J. 1997. The environmental fate of phthalate esters: a literature review. *Chemosphere*. 35 (4): 667-749. doi: 10.1016/S0045-6535(97)00195-1.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Sullivan, S. and Teague, J.L. 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ. Health Perspect*. 113: 1056-1061. doi: 10.1289/ehp.8100.

Tan, W., Zhang, Y., He, X., Xi, B., Gao, R., Mao, X., Huang, C., Zhang, H., Li, D., Liang, Q., Cui, D. and Alshawabkeh, A.N. 2016. Distribution patterns of phthalic acid esters in soil particle-size fractions determine biouptake in soil-cereal crop systems. *Scientific Report*. 6 : 31987.

Tran, B.C., Teil, M.J., Blanchard, M., Alliot, F. and Chevreuil, M. 2015. Fate of phthalates and BPA in agricultural and non-agricultural soils of the Paris area (France). *Environ. Sci. Pollut. Res*. 22 : 11118-11126. doi: 10.1007/s11356-015-4178-3.

UBA, 2012a. Umweltbundesamt. Schriftenreihe Umwelt und Gesundheit: Band I: Phthalat - Belastung der Bev¨olkerung in Deutschland: Expositions relevante Quellen, Aufnahmepfade und Toxikokinetik am Beispiel von DEHP und DINP. Berlin.

UBA. 2012b. Umweltbundesamt. Schriftenreihe Umwelt and Gesundheit: Phthalat – Belastung der Bev¨olkerung in Deutschland: Expositions relevante Quellen, Aufnahmepfade und Toxikokinetik am Beispiel von DEHP und DINP – Kurzfassung and Summary. Berlin.

USEPA. 1997. US Environmental Protection Agency. Integrated risk information system: di (2-ethylhexyl) phthalate (DEHP) (CASRN 117-81-7): Chemical Assessment Summary, National Centre for Environmental Assessment.

Wams, T.J. 1987. Diethylhexylphthalate as an environmental contaminant-a review. *Sci. Total Environ.* 66 : 1-16. doi: 10.1016/0048-9697(87)90072-6.

Wang, L., Liu, M., Tao, W., Zhang, W., Wang, L., Shi, X., Lu, X. and Li, X.P. 2018. Pollution characteristics and health risk assessment of phthalate esters in urban soil in the typical semi-arid city of Xi'an, Northwest China. *Chemosphere*. 191 : 467-476.

Wang, X., Lin, Q., Wang, J., Lu, X. and Wang, G. 2013. Effect of wetland reclamation and tillage conversion on accumulation and distribution of phthalate esters residues in soils. *Ecological Engineering.* 51 : 10-15.

Wormuth, M., Scheringer, M., Vollenweider, M. and Hungerbuhler, K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal*. 26 (3) : 803-824. doi: 10.1111/j.1539-6924.2006.00770.x.

Wu, W., Hu, J., Wang, J., Chen, X., Yao, N., Tao, J. and Zhou, Y.K. 2015. Analysis of phthalate esters in soils near an electronics manufacturing facility and from a non-industrialized area by gas purge microsyringe extraction and gas chromatography. *Science of The Total Environment*. 508 : 445-451.

Zeiger, E., Haworth, S., Speckt, W. and Mortelmans, K. 1982. Phthalate Ester Testing in the National Toxicology Program's Environmental Mutagenesis Test Development Program. *Environmental Health Perspectives.* 45 : 99-101.