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Research Article

Determination of metformin and triclosan in sewage sludge using Liquid chromatography-mass spectrometry (LC-MS)

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Abstract
Pharmaceuticals and personal care products (PPCPs) are generally neither totally removed by sewage treatment nor completely destroyed in the environment. Metformin (MET) and triclosan (TRI) are two compounds in PPCPs that have the potential to be environmental pollutants. This research aimed to determine MET and TRI in sewage sludge using a liquid chromatograph-mass spectrometer (LCMS-8040) and a sewage sludge extraction method. The Milli-Q water and sewage sludge were also tested at three different MET and TRI concentrations (0.01, 0.02, and 0.03 mg L^{-1}). As a result, the corresponding recoveries of MET and TRI in both matrixes ranged from 85.93 to 116.10 per cent and 90.50 to 116.30 per cent (n = 7, RSD < 10%). Then, the limit of detection (LOD) and the limit of quantification (LOQ) for MET and TRI were found to be 0.005 and 0.01 mg L^{-1}. The amounts of MET and TRI in the sewage sludge samples from the Ukkadam sewage treatment plant (USTP), Coimbatore, ranged from BDL to 0.0587 mg L^{-1} and 0.0719 to 0.1851 mg L^{-1}, respectively. Consequently, the amounts of MET and TRI in the sewage sludge samples from the Tamil Nadu Agricultural University sewage treatment plant (TSTP), Coimbatore, ranged from BDL to 0.0227 mg L^{-1} and 0.0393 to 0.1296 mg L^{-1}, respectively. This exclusive use of the highly sensitive LCMS-8040 consumes less time than other analytical methods for measuring the amount of MET and TRI in sewage sludge by overcoming the risk of chemical degradation.

Keywords: Sewage sludge, Metformin (MET), Triclosan (TRI), Liquid chromatography-mass spectrometry (LC-MS)

INTRODUCTION

One of the biggest issues harming our planet is chemical pollution. It is a cyclical process that impacts all forms of the environment (air, water, and soil), as well as living things that are both pollutant emitters and recipients. Due to their design to remove organic debris and nutrients at concentrations higher than mg L^{-1}, one of the main issues is that wastewater treatment plants (WWTPs) are unable to remove many EPs (Pérez-

Lemus *et al*., 2020). Wastewater treatment plants produce significant sewage sludge (Li *et al*., 2018). Currently, the five main methods of disposing of sewage sludge are landfill, land application, drying-incineration, composting, and recycling as building materials (Guo *et al*., 2020). A viable technique for getting rid of sewage sludge is land application (Verlicchi and Zambello, 2015). However, pathogens, inorganic (such heavy metals) and organic hazardous contaminants in sewage sludge restrict its use as a soil amendment (Fijalkowski *et al*., 2017; Dubey *et al*., 2021). The presence of pharmaceuticals and personal care products (PPCPs) in sewage sludge has also drawn increasing attention (Ezzariai *et al*., 2018; Guo *et al*., 2020). Both untreated and treated sludge (such as digested, dewatered, conditioned, and dried sludge) included a variety of PPCP chemicals, with concentrations ranging from 10^{-1} to 10^{5} ng g^{-1} (for example, 152 pharmaceuticals and 17 personal care products) . For instance, the main sludge included triclosan (133 ng g^{-1}) (Verlicchi and Zambello, 2015). Thus, it is imperative to provide methods for controlling and lowering PPCP levels. Sludge applied to the land can increase the levels of these pollutants in the soil, runoff, and groundwater (Kim *et al*., 2017). As a result, sewage sludge must have reduced amounts of dangerous contaminants. Composting is a practical method for removing organic contaminants in sewage sludge (Ezzariai *et al*., 2018; Guo *et al*., 2020).

The first-line drug for treating type 2 diabetes is metformin (MET) (Tee *et al*., 2022). According to the most recent data from the International Diabetes Federation, 536.6 million persons aged 20 to 79 had diabetes in 2021, making up approximately 10.5% of the total population in this age group (IDF Diabetes Atlas, 10th edition, 2021). According to the IDF, there will be dangerously more people with diabetes by the years 2030 and 2045, reaching 642.7 million and 783.2 million, respectively. MET does not undergo human metabolization like many pharmaceutical medications do; instead, it moves through the body unaltered. So, 70 percent of it is eliminated in urine and the remaining 30 percent in faeces after consumption. MET has been identified as a major contaminant contributing to significant mass loads in WWTPs (Briones and Sarmah, 2019; Bhowmick *et al*., 2022). During sewage treatment, MET concentrations are significantly reduced, mainly due to microbial degradation. Despite the high removal efficiency of sewage treatment plants (STPs), MET is still released in significant amounts into the aquatic environment (Bhowmick *et al*., 2022). Since it is not naturally degraded, MET is easily reintroduced to humans as they move up the food chain (Meffe *et al*., 2021). Barros *et al*. (2022) showed that MET behaves as an endocrine disruptor at environmentally relevant quantities. MET is a mobile compound with a low affinity to soils (Mrozik & Stefanska, 2014). Once introduced into watercourses, MET remained tenacious and non-biodegradable with a higher eco-toxicity index due to its exceptionally low K_{OW} (Octanol/water partition coefficient) value (-1.83) and strong aqueous mobility (Li and Tabassum, 2021; Bhowmick *et al*., 2022). This indicates that this drug may potentially threaten ground and surface water (Haiba et al., 2017).

Triclosan (TRI) is a phenyl ether (5-chloro-2-(2,4-dichlorophenoxy) phenol) with anti-microbial and anti-fungal properties (Montaseri and Forbes, 2016). The use of triclosan-containing personal care items like cleaning materials, toothpaste, deodorants, antibacterial soap, skin lotion, and disinfectants may be the cause of the presence of triclosan in Indian sewage water (Motia *et al*., 2019; Kumar *et al*., 2021; Wang *et al*., 2021). The content of TRI in personal care products should be below 0.3% of product mass as directed by the US Food and Drug Agency (USFDA) and the European Community Cosmetic Directive (Dhillon *et al*., 2015). TRI has been found in a wide range of habitats, including surface water, river sediment, and biosolids (Wang and Liang, 2021). TRI would stay in the environment after entering through WWTP effluent and sewage sludge due to its hydrophobicity, durability, and biological accumulation capacity, with a K_{OW} value of 4.6. TRI may have hazardous biochemical effects on species and affect the environment by interfering with the biochemical cycle, ecosystem structure and organism function at higher concentrations (e.g., 1.4 g L^{-1}). Endocrine disruption, antibiotic resistance, the production of carcinogenic products, and allergies are just a few of the health issues linked to TRI (Xin *et al*. 2021).

The data about the presence of MET and TRI in sewage sludge is scarce in India. The LC-MS approach, which can perform both positive and negative ionisation, has the best sensitivity of any spectroscopic or chromatographic technology and enables thorough, high-throughput analysis. Moreover, the capacity of LCMS to identify and analyse a wider spectrum of compounds has allowed for the development of extraction processes that are quicker and less time-consuming (Pérez-Lemus *et al*., 2022). This study aimed to determine the amount of MET and TRI residues in sewage sludge using a suitable extraction method and LC-MS.

MATERIALS AND METHODS

Chemicals and materials

Metformin hydrochloride and triclosan (Isotopically labelled external standards), formic acid (purity ≥ 98%), as well as LC-MS grade methanol and acetonitrile, were purchased from Sigma-Aldrich (Bangalore, India) with a purity of ≥ 99 %. Methanol was used as a solvent to prepare a combined standard stock solution (100 mg L^{-1}) and then stored at -10°C. The calibration before each analysis was done using freshly prepared working

standard solutions. Milli-Q water (18.1 MΩ•cm at 25 °C) which has a total organic carbon (TOC) level of ≤ 0.005 mg L^{-1}) was utilised as a mobile phase for LC-MS analysis and also used for the experiments.

Sample collection

The sewage sludge samples were collected from a municipal wastewater treatment plant in Ukkadam with a treatment capacity of 70 million litres per day and Tamil Nadu Agricultural University, a treatment capacity of 200 m^3 per day, Coimbatore, Tamil Nadu. The grab samples (Once a month from April 2021 to March 2022) of sewage sludge were collected from the sampling locations (TSTP - 11°00'27.6"N, 76°56'04.1"E and USTP - 10°59'01.6"N, 76°58'22.7"E) respectively and homogenized before analysis.

Sewage sludge extraction method

The sewage sludge extraction method followed was slightly modified by Haiba *et al*. (2018). The sewage sludge samples (5g) were precisely weighted into 50 mL polypropylene centrifuge tube. 20 mL of extraction solvent (1% v/v formic acid in ethanol) was added to a sample tube and vortex mixed (Chiltren Scientific MT 17 vortex mixer) for 1 min. The sample tube was tightly capped and placed horizontally on a shaker (200 rpm) for 15 min. The tube was turned into a vertical position and shaken manually to ensure that the solid content was in contact with the extraction solvent. It was continued by sonicating for about 20 min. Then, the samples were centrifuged (high-speed chilled centrifuge - REMI) at 6000 rpm for 10 min. The extracts were removed from the tube using a pipette. These steps were repeated five times with each sample. Extracts were combined in 100 mL polypropylene bottles, mixed and weighted. From each extract 15 mL was taken into 50 mL polypropylene centrifuge tube for further treatment. Prior to LC-MS analysis, sample extracts were diluted: to 100 µL extract, 1400 µL of milliQ water were added in 1.5 mL Eppendorf tube. Automatic pipette was used for dosing, but all the solutions were weighted. The solutions were vortex-mixed and filtered through a syringe filter. First five drops of the filtrate were discarded and the remaining (1 mL) was collected into autosampler vial (1.8 mL glass vial).

Calibration and quality control samples

Calibration and quality control samples were prepared by diluting stock solutions of analytes. Stock solutions were prepared by dissolving an appropriate amount of analytes in methanol. MET (100 mg L^{-1}) and TRI (100 mg L^{-1}) were prepared in methanol as a combined stock standard solution. Subsequently, it was serially diluted to produce the working standard solutions of 0.01, 0.02, 0.03, 0.04 and 0.05 mg L^{-1} in methanol for the calibration of the LC-MS. Similarly to the preparation of sample solutions, all solutions were prepared by weight, vortex-mixed and filtered through syringe filters. The concentration of calibration and quality control solutions were chosen according to the linear range for each analyte.

LC-MS methodology

All analysis was performed on an LC-MS – 8040 (Shimadzu UFLC – LC-20 AD) coupled with an electrospray ionization source (ESI) which has a triple quadrupole mass spectrometer. The LC-MS conditions required for the analysis are displayed in Table 1. The low-pressure gradient was used to elute the metformin and triclosan linearly from 30% B (for 2 minutes) to 98 percent B in 6 min (2 min hold) and back to 30% B in 10 min (5 min hold). The total run time of the linear gradient was about 15 minutes. Additionally, the conditioning of the column was done for 5 minutes. MET and TRI were estimated using retention time-dependent scheduled multiple reaction monitoring

Table 1. LC-MS analysis conditions

LC conditions	
Instrument	LCMS – 8040 (Shimadzu UFLC – LC-20 AD)
Autosampler	SIL-20AC HT
Communication Bus Module	CBM – 20 A
Mode	Low pressure gradient
Column oven	CTO-20AC
Column	Shimadzu Shim pack GISS C18 column with dimensions of 4.6 × 250 mm, 5 µm.
Column temperature	40 °C ± 1°C
Mobile phase	Milli-Q water LCMS grade methanol 0.5 % formic acid in both mobile phases
Flow rate	0.8000 mL/min
Injection volume	0.01 Ml
Post time	5 minutes
Gradient elution profile	0–2 minutes: 30 % B; 2–6 minutes: 30 - 98 % B; 6–8 minutes: 98 %;8–10 minutes: 98 - 30 %; 10 – 15 minutes: 30 %
Total run time	15 minutes
MS conditions	
Interface	Electrospray Ionisation (ESI) with triple quadrupole mass spectrometer
Interface current	0.1 uA
Ionization mode	Positive and Negative
Desolvation Line (DL) temperature	250 °C
CID gas pressure	230 kPa
Drying gas flow rate	15 L min^{-1}
Nebulizing gas flow	2.5 L min^{-1}
Heat block temperature	400 °C

(MRM) with two distinct mass transitions: one as the qualifier ion and the other as the quantifier ion. The MRM detection window was 1 min, with a target scan time of 0.206 s. According to the SANTE/11945/2015 recommendations, the ratio of two mass transitions (m/z ion ratio) was used to confirm both the analytes. Data acquisition and system management were performed using the lab solution software. The LC-MS parameters were optimised for the quantification of both the analytes and the method was validated after the sample preparation methodology.

RESULTS AND DISCUSSION

Optimization of the Mass Spectrometer (MS) and method application

First, the standard solutions of metformin and triclosan were subjected to a Q1-MS scan in both positive and negative ionisation modes, identifying the proper precursor ions. The identified precursor ions were then subjected to the scanning for product ions. Then, by fine-tuning the precursor transmission and fragmentation parameters, the respective transmissions of 130/60.20 and 289/34.80 were used to measure MET and TRI. The other ion transmissions (130/71.10, 289/35.00) were used for the qualitative confirmation. Table 2 and Fig. 1 display the mass spectrometric characteristics and MRM events of MET and TRI, respectively.

MET and TRI were separated using Milli-Q water and methanol (each with 0.5 percent formic acid) as the mobile phases. Shimadzu Shim pack GISS C18 column (4.6 × 250 mm, 5 µm) was used with the MRM acquisition parameter. Fig. 2 shows the MRM chromatograms for MET and TRI during the course of 15-minute gradient elution, respectively, with retention periods of 3.624 and 11.518 minutes. During the examination of the sewage sample, the leftover matrix of the sample following cleanup could obstruct the process and shorten the column's life. In order to ensure complete column cleaning between analyses, the gradient profile was changed to pure methanol for five minutes after both analytes had eluted from the column.

The varying pH of the mobile phase has an impact on the column stationary phase, column temperature, column length, particle size, mobile phase solvent(s), buffer pH, and ionisation of the target analytes; it could also affect resolution. Resolution and column efficiency are both influenced by peak symmetry (Marie *et al*., 2022). The less polar TRI was studied in the negative-polarity mode, whilst the highly polar MET was evaluated in the positive-polarity mode (Kaur *et al*., 2021). As a result, the linear gradient mode was able to achieve appropriate retention and separation for the MET and TRI (Kachhawaha *et al*., 2017). It was buffering greatly enhanced the recoveries of MET and TRI. Since the buffering agents improved the partitioning of the target analytes into the organic phase, this was explicable (acetonitrile). It was crucial to modify the pH to get sufficient intensities for all of these chemicals and obtain acceptable recoveries for these compounds. The pH of the aliquot produced from the extraction was altered to pH 7 before evaporation since the recoveries of these analytes were improved at pH 7.

Table 2. Summary of metformin and triclosan mass spectrometric characteristics

Analyte	ESI	Molecular weight	Q1	Q3	CE	Q3*	CE*	RT (min)
Metformin	+	129.20	130.00	60.20	-16	71.10	-23	3.624
Triclosan	-	289.00	289.00	34.80	35	35.00	16	11.518

Q1- Precursor ion, Q3- Quantifier product ion, Q3* - Qualifier product ion, CE- Collision Energy, CE* - Collision Energy for Q3*, RT- Retention time

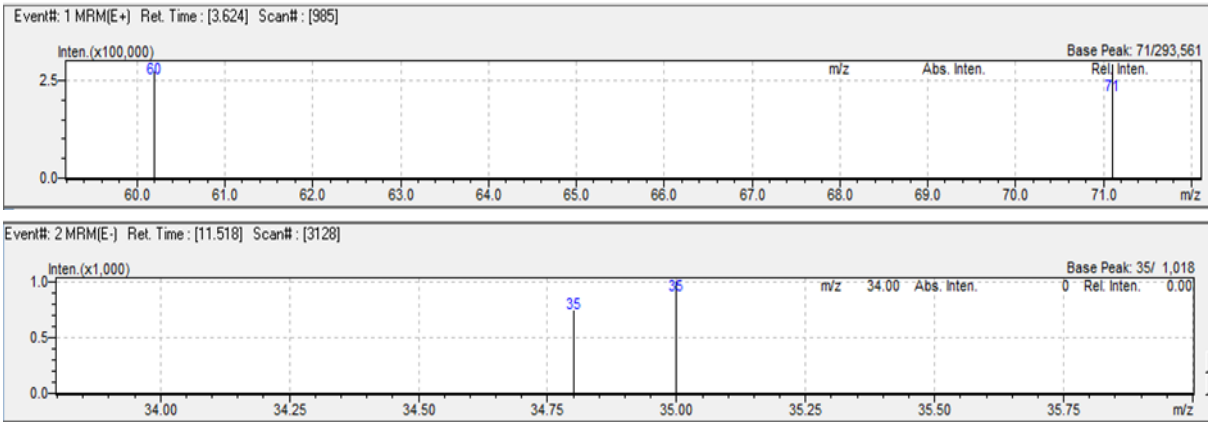

Fig. 1. *MRM events for the ionisation of metformin and triclosan*

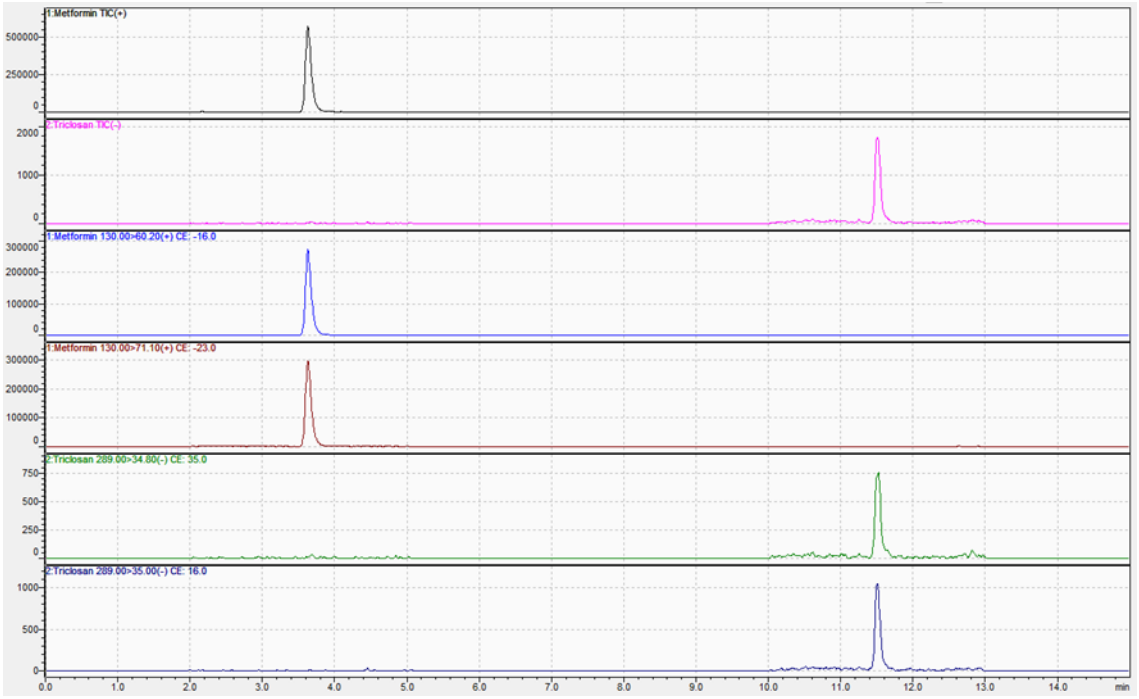

Fig. 2. *MRM chromatograms for MET and TRI in positive and negative ionisation modes*

The sensitivity of the method was proved by the spiking levels of metformin and triclosan in Milli-Q water and sewage sludge. The recoveries of the MET and TRI in Milli-Q water spiked at 0.01, 0.02 and 0.03 mg L^{-1} ranged from 103.77 to 116.10 % and from 112.87 to 116.30 % (n=7), respectively. In actuality, at the same level, the recoveries of TRI and MET in sewage sludge ranged from 90.5 to 114.6 % and 85.93 to 94.20 % (n=7), respectively. The results show that the technique generally operates within an acceptable range of acceptable recovery (70-120%) and precision (RSD<10%). MET and TRI had LODs and LOQs of 0.005 and 0.010 mg L^{-1}, respectively. These results demonstrated that the developed method could be used to detect TRI and MET residues. The validation results are summarized in Table 3. The matrix effect in sewage sludge was also noted (< 10 %).

Quantification of MET and TRI in sewage sludge

This sewage sludge extraction method and LC-MS method have been successfully used over the sewage sludge samples for the quantification of MET and TRI. The concentrations of MET and TRI in sewage samples are shown in Fig. 3 and 4. Metformin was detected in sewage sludge of USTP and TSTP in the range of BDL to 0.0587 mg L^{-1} and BDL – 0.0227 mg $L^{-1,}$ respectively. Likewise, the sewage sludge of the USTP and TSTP had respective triclosan concentrations of BDL - 0.1851 mg L^{-1} and BDL - 0.1296 mg L^{-1} (with RSDs 10%, n=6). This preliminary dataset shows the presence of triclosan and metformin in Indian sewage waters. This may be due to high polarity and metformin stability, making it difficult for the body to break it down (Kachhawaha *et al*., 2021). The quick adsorption of MET and TRI (from the liquid phase) to the solid sewage sludge particles was likely the cause of all concentration readings. This is consistent with the information provided in earlier research (Nei *et al*., 2014).

Conclusion

The present study concluded that there was substantial proof of the presence of MET and TRI in sewage sludge of USTP and TSTP, Coimbatore, Tamil Nadu.

Table 3. Validated parameters in Milli-Q water and sewage sludge

Analyte	LOD (mg L^{-1})	LOQ (mg L^{-1})	Milli-Q water Recovery (%) (n=6 and RSD < 10 %)			Sewage sludge Recovery (%) (n=6 and RSD < 10 %)		
			0.01 mg L^{-1}	0.02 mg L^{-1}	0.03 mg L^{-1}	0.01 mg L^{-1}	0.02 mg L^{-1}	0.03 mg L^{-1}
Metformin	0.005	0.01	110.80	116.10	103.77	94.20	90.62	85.93
Triclosan	0.005	0.01	116.30	114.90	112.87	114.60	99.20	90.50

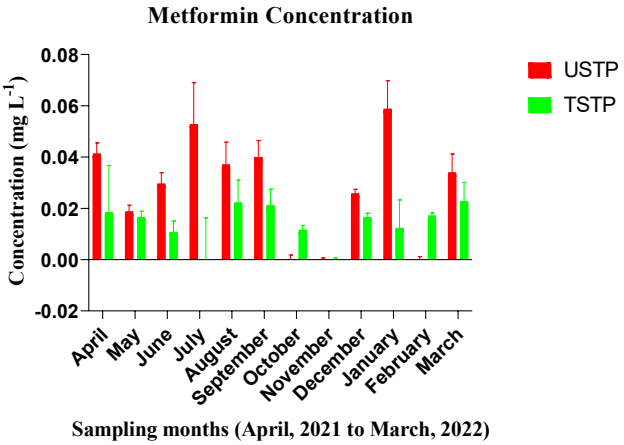

Fig. 3. *MET concentration in USTP and TSTP*

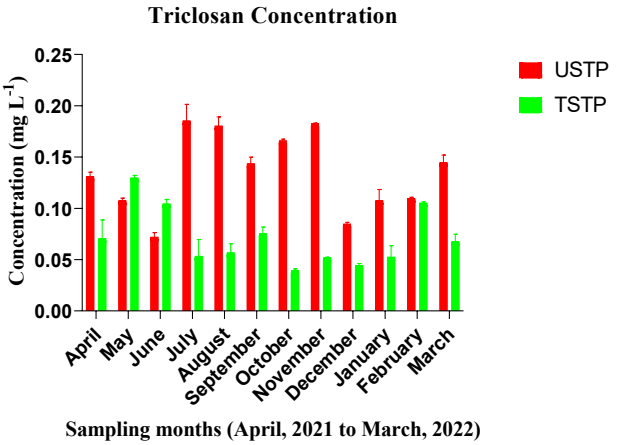

Fig. 4. *TRI concentration in USTP and TSTP*

Also, the established sludge extraction and LC-MS methods for the quantification of MET and TRI is trustworthy and reproducible with desired recovery rates (70 -120%, n=7, RSD < 10%), matrix effect (< 10%), LOD (0.005 mg L^{-1}) and LOQ (0.01 mg L^{-1}). Hence, this method could be used for routine monitoring as well as regulatory purposes. It also demonstrated that these concentrations of MET and TRI in sewage sludge might move into the food chain after the land application, causing ecotoxicity. To further understand the causes of accelerating MET and TRI degradation during composting of sewage sludge, more research is required. Given the paucity of studies on the presence of MET and TRI in Indian sewage water, this work may serve as a baseline study and serve as an impetus for further MET and TRI research in India.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Barros, S., Ribeiro, M., Coimbra, A. M., Pinheiro, M., Morais, H., Alves, N. & Neuparth, T. (2022). Metformin disrupts *Danio rerio* metabolism at environmentally relevant concentrations: A full life-cycle study. *Science of The Total Environment*, 157361. https://doi.org/10.1016/j.scitotenv.2022.157361.
2. Briones, R. M. & Sarmah, A. K. (2019). Modelling degradation kinetics of metformin and guanylurea in soil microcosms to derive degradation end-points. *Environmental Pollution*, *245*, 735-745.https://doi.org/10.1016/j.envpol.2018.11.045.
3. De Bhowmick, G., Briones, R. M., Thiele-Bruhn, S., Sen, R. & Sarmah, A. K. (2022). Adsorptive removal of metformin on specially designed algae-lignocellulosic biochar mix and techno-economic feasibility assessment. *Environmental Pollution*, *292*, 118256. https://doi.org/10.1016/j.envpol.2021.118256.
4. Dhillon, G. S., Kaur, S., Pulicharla, R., Brar, S. K., Cledón, M., Verma, M. & Surampalli, R. Y. (2015). Triclosan: current status, occurrence, environmental risks and bioaccumulation potential. *International Journal of Environmental Research and Public Health*, *12*(5), 5657-5684. https://doi.org/10.3390/ijerph120505657.
5. Dubey, M., Mohapatra, S., Tyagi, V. K., Suthar, S. & Kazmi, A. A. (2021). Occurrence, fate, and persistence of emerging micropollutants in sewage sludge treatment. *Environmental Pollution*, *273*, 116515. https://doi.org/10.1016/j.envpol.2021.116515.
6. Ezzariai, A., Hafidi, M., Khadra, A., Aemig, Q., El Fels, L., Barret, M., ... & Pinelli, E. (2018). Human and veterinary antibiotics during composting of sludge or manure: Global perspectives on persistence, degradation, and resistance genes. *Journal of Hazardous Materials*, 359, 465-481. https://doi.org/10.1016/j.jhazmat.2018.07.092.
7. Fijalkowski, K., Rorat, A., Grobelak, A., & Kacprzak, M. J. (2017). The presence of contaminations in sewage sludge –The current situation. *Journal of Environmental Management*, *203*, 1126-1136. https://doi.org/10.1016/j.jenvman.2017.05.068
8. Guo, Y., Rene, E. R Guo, Y., Rene, E. R., Wang, J. & Ma, W. (2020). Biodegradation of polyaromatic hydrocarbons and the influence of environmental factors during the co-composting of sewage sludge and green forest waste. *Bioresource Technology*, *297*, 122434. https://doi.org/10.1016/j.biortech.2019.122434.
9. Haiba, E., Nei, L., Herodes, K., Ivask, M. & Lillenberg, M. (2018). On the degradation of metformin and carbamazepine residues in sewage sludge compost. http://dx.doi.org/10.15159/ar.18.123.
10. Haiba, E., Nei, L., Kutti, S., Lillenberg, M., Herodes, K.,

Ivask, M. & Laaniste, A. (2017). Degradation of diclofenac and triclosan residues in sewage sludge compost. *Agronomy Research*, *15*(2), 395-405.
11. International Diabetes Federation. (2021). IDF Diabetes Atlas 10th ed.
12. Kachhawaha, A. S., Nagarnaik, P. M., Jadhav, M., Pudale, A., Labhasetwar, P. K. & Banerjee, K. (2017). Optimization of a modified QuEChERS method for multi-residue analysis of pharmaceuticals and personal care products in sewage and surface water by LC-MS/MS. *Journal of AOAC International*, *100*(3), 592-597. https://doi.org/10.5740/jaoacint.17-0060.
13. Kachhawaha, A. S., Nagarnaik, P. M., Labhasetwar, P. K. & Banerjee, K. (2021). Pharmaceuticals and personal care products in aqueous urban environment of western India. *Water and Environment Journal*, *35*(4), 1302-1312. https://doi.org/10.1111/wej.12720
14. Kaur, G., Garg, S., Sharma, P. & Sud, D. (2021). A Review on High Performance Liquid Chromatographic Methods for the Determination of Metformin. *Current Analytical Chemistry*, *17*(6), 754-767. https://doi.org/10.2174/1573411016666200310141939.
15. Kim, M., Li, L. Y., Gorgy, T. & Grace, J. R. (2017). Review of contamination of sewage sludge and amended soils by polybrominated diphenyl ethers based on meta-analysis. *Environmental Pollution*, *220*, 753-765. https://doi.org/10.1016/j.envpol.2016.10.053.
16. Kumar, S., Paul, T., Shukla, S. P., Kumar, K., Karmakar, S., & Bera, K. K. (2021). Biomarkers-based assessment of triclosan toxicity in aquatic environment: A mechanistic review. *Environmental Pollution*, *286*, 117569. https://doi.org/10.1016/j.envpol.2021.117569.
17. Li, J. & Tabassum, S. (2021). Influence and efficiency of an anti-diabetic drug Metformin hydrochloride as an uncoupler: Reducing sludge production. *Cleaner Engineering and Technology*, *4*, 100203. https://doi.org/10.1016/j.clet.2021.100203.
18. Li, J., Yu, G., Xie, S., Pan, L., Li, C., You, F. & Wang, Y. (2018). Immobilization of heavy metals in ceramsite produced from sewage sludge biochar. *Science of the Total Environment*, *628*, 131-140. https://doi.org/10.1016/j.scitotenv.2018.02.036
19. Marie, A. A., Salim, M. M., Kamal, A. H., Hammad, S. F. & Elkhoudary, M. M. (2022). Analytical quality by design based on design space in reversed-phase-high performance liquid chromatography analysis for simultaneous estimation of metformin, linagliptin and empagliflozin. *Royal Society Open Science*, *9*(6), 220215. https://doi.org/10.1098/rsos.220215
20. Meffe, R., de Santiago-Martín, A., Teijón, G., Hernández, V. M., López-Heras, I., Nozal, L. & de Bustamante, I. (2021). Pharmaceutical and transformation products during unplanned water reuse: Insights into natural attenuation, plant uptake and human health impact under field conditions. *Environment International*, *157*, 106835. https://doi.org/10.1016/j.envint.2021.106835
21. Montaseri, H. & Forbes, P. B. (2016). TrAC trends, *Anal. Chem*, *85*, 221.
22. Motia, S., Tudor, I. A., Ribeiro, P. A., Raposo, M., Bouchikhi, B. & El Bari, N. (2019). Electrochemical sensor based on molecularly imprinted polymer for sensitive triclosan detection in wastewater and mineral water. *Science of the Total Environment*, *664*, 647-658. https://doi.org/10.1016/j.scitotenv.2019.01.331.
23. Mrozik, W. & Stefańska, J. (2014). Adsorption and biodegradation of antidiabetic pharmaceuticals in soils. *Chemosphere*, *95*, 281-288. https://doi.org/10.1016/j.chemosphere.2013.09.012.
24. Nei, L., Haiba, E., Kutti, S., Kipper, K., Herodes, K. & Lillenberg, M. (2014). Sewage sludge compost, microbial activity and pharmaceuticals. *Global Journal on Advances Pure and Applied Sciences*, *3*.
25. Pérez-Lemus, N., López-Serna, R., Pérez-Elvira, S. I. & Barrado, E. (2022). Analysis of 60 pharmaceuticals and personal care products in sewage sludge by ultra-high performance liquid chromatography and tandem mass spectroscopy. *Microchemical Journal*, *175*, 107148. https://doi.org/10.1016/j.microc.2021.107148
26. Pérez-Lemus, N., López-Serna, R., Pérez-Elvira, S. I. & Barrado, E. (2020). Sample pre-treatment and analytical methodology for the simultaneous determination of pharmaceuticals and personal care products in sewage sludge. *Chemosphere*, *258*, 127273. https://doi.org/10.1016/j.chemosphere.2020.127273.
27. Tee, K. B., Ibrahim, L., Hashim, N. M., Saiman, M. Z., Zakaria, Z. H. & Huri, H. Z. (2022). Pharmacokinetic–Pharmacometabolomic Approach in Early-Phase Clinical Trials: A Way Forward for Targeted Therapy in Type 2 Diabetes. *Pharmaceutics*, *14*(6), 1268. https://doi.org/10.3390/pharmaceutics14061268.
28. Verlicchi, P. & Zambello, E. (2015). Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil—A critical review. *Science of the Total Environment*, *538*, 750-767. https://doi.org/10.1016/j.scitotenv.2015.08.108
29. Wang, D., Yi, N., Wang, Y., Yang, J., Fu, Q., Liu, X. & Ni, B. J. (2021). Triclosan degradation in sludge anaerobic fermentation and its impact on hydrogen production. *Chemical Engineering Journal*, *421*, 129948. https://doi.org/10.1016/j.cej.2021.129948.
30. Wang, Y. & Liang, W. (2021). Occurrence, toxicity, and removal methods of triclosan: a timely review. *Current Pollution Reports*, *7*(1), 31-39. https://doi.org/10.1007/s40726-021-00173-9
31. Xin, X., Huang, G., Zhang, B. & Zhou, Y. (2021). Trophic transfer potential of $nTiO_2$, nZnO, and triclosan in an algae-algae eating fish food chain. *Aquatic Toxicology*, *235*, 105824. https://doi.org/10.1016/j.aquatox.2021.105824.