



Bioremediation of carbendazim and thiamethoxam in domestic greywater using a bioaugmented microbial consortium

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ABSTRACT

The safe and effective removal of pesticides remaining in environmental media such as greywater is important because of their potential hazard. In this study, the feasibility of a bioremediation approach has been studied against two pesticides (i.e., carbendazim and thiamethoxam) based on three microbial formulations: (1) a fungus culture, (2) an isolated microbial consortium, and (3) an augmented microbial consortium with fungus. The toxicity of the two pesticides in ground water was assessed in terms of the attenuation factor index and the environmental potential risk indicator for pesticides. The concentrations of each pesticide in greywater matrix were measured using an automated isotope dilution solid phase extraction-liquid chromatography-tandem mass spectrometry. The efficiency of bioaugmented microbial consortium, when assessed for carbendazim and thiamethoxam in terms of the extent of degradation over 10 days, was 94.4 and 93.6%, respectively. Two types of toxicity indicators (i.e., environmental potential risk indicator for pesticides (EPRIP) and attenuation factor index (AFI)) were computed to assess the environmental relevancy of target pesticides in surface and ground waters. Accordingly, their values for carbendazim (EPRIP of 42.1 and AFI of 1003.4) were higher than those of thiamethoxam (EPRIP of 6.4 and AFI of 1001.2). The bacterial culture augmented with fungus *Aspergillus versicolor* is thus found to be a promising tool to enhance the removal efficiency of the toxic pesticides present in biosystems. It is expected that this study will help promote the research associated with bioaugmentation for diverse microbial species (e.g., *Pseudomonas*, *Klebsiella species*, and *Bacillus subtilis*) with fungus for enhanced biodegradation processes.

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1. Introduction

The demand of greywater recycling for non-contact use has been increasing steadily with the rapid growth of population. Greywater in domestic residential areas (e.g., non-toilet plumbing sources including kitchens, basins, and

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bathrooms) is less polluted than municipal wastewater because it does not contain feces, urine, and toilet paper. Therefore, greywater can be regarded as a recyclable water resource to meet the demand for freshwater (Shaikh and Ahammed, 2020). Treated greywater can also be reused for toilet flushing and irrigation of non-food producing plants (Rodríguez et al., 2021). According to the World Health Organization (WHO), greywater with a low pathogenic content can be used for crop irrigation (Hai et al., 2012). However, proper evaluation of greywater is crucial prior to its recycling and/or reuse since it can contain harmful chemical residues (e.g., pesticides) (Noman et al., 2019). Pesticides in greywater can accumulate in a certain environmental reservoir and penetrate into the human food chain (Kim and Zoh, 2016). Carbenazim (CBZ) and thiamethoxam (THX) are common pesticides used as fungicide and insecticide, respectively (Carazo-Rojas et al., 2018). CBZ is used to control plant diseases and soilborne diseases in a variety of crops whereas THX is used to kill sucking and chewing insects that feed on roots, leaves, and other plant tissues. They can be readily released into greywater while washing fruits and vegetables (Liu et al., 2018). The serious concerns on their environmental hazardousness are associated with their long-term persistency (e.g., ~3 years) in surface water (de Souza et al., 2020). Therefore, treatment of such pesticides from greywater samples is essential to reduce their environmental and human health risks (Bonnechère et al., 2012).

Bioremediation with specific bacterial or fungal species is an efficient option for degrading pesticides (Nie et al., 2020). Among such options, an approach based on individual strains and a consortium of strains has been widely studied (Li et al., 2020). Fungi are the preferred media for bioremediation processes because they possess enzymes can use organic contaminants as carbon feedstock (Maqbool et al., 2016). According to the Pareto's law, a small portion of organisms burns a majority of the energy flux (Medaura et al., 2021). Hence, fungi are considered to be suitable inoculants for bioaugmentation. Furthermore, fungi are stable and adaptable to environmental changes (Hassan et al., 2020). Several studies have reported on the removal of pesticides from industrial and municipal wastewater using microbes (Fang et al., 2018; Abdel-Razek et al., 2019; Zhang et al., 2020). It should be noted that there have not been many reports on the degradation of pesticides over bioaugmented fungus inoculated into a naturally derived bacterial consortium (Castro-Gutiérrez et al., 2019). Furthermore, the development of novel treatment techniques to remove pesticides from contaminated greywater has rarely been investigated.

The purpose of this study is to develop an efficient bioaugmented system for the degradation of CBZ and THX in greywater. In nature, indigenous consortium found in kitchen waste sludge is found to degrade organics for its growth, but at very slow pace. In this context, bioaugmentation of slow growing microbes with other microorganism is regarded as an emerging technique for fastening the biodegradation processes. In the present work, an attempt was made to realize the synergistic bioaugmentation effects between the derived bacterial consortium and *Aspergillus versicolor* (*A. versicolor*) for enhanced biodegradation of pesticides in greywater. The environmental relevance of the target pesticides and their mobility potentials were evaluated in terms of the environmental potential risk indicator for pesticides (EPRIP) (Aravinna et al., 2017) and the attenuation factor index (AFI) in order to evaluate their noxious effects on surface water and groundwater. To the best of the authors' knowledge, this was the first effort to describe the potential utility of an augmented microbial consortium of *A. versicolor* in the bioremediation of pesticides in greywater.

2. Materials and methods

2.1. Reagents and chemicals

High purity analytical grade pesticide reagents of CBZ ($C_9H_9N_3O_2$) and THX ($C_8H_{10}ClN_5O_3S$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hyper-grade ethyl acetate ($C_4H_8O_2$) and dichloromethane (CH_2Cl_2) were obtained from Rankem (India). Hyper-grade methanol (CH_3OH) was purchased from J.T. Baker (India). Anhydrous sodium sulfate (Na_2SO_4 ; M.W. = 142.04 g/mol), dipotassium phosphate (K_2HPO_4 ; M.W. = 174.2 g/mol), sodium hydroxide (NaOH; M.W. = 39.99 g/mol), and hydrochloric acid (HCl; M.W. = 36.46 g/mol) were supplied by Sd Fine Chemicals (Mumbai, India). Deionized (DI) water made using a Milli-Q system (Millipore, Tokyo, Japan) was used to treat or prepare all samples.

2.2. Greywater sampling

Residential greywater samples (excluding toilet wastewater) were collected in June 2017 during 13:00–13:15 IST from household drains in the Whitefield locality of Bangalore (between $12^\circ 58'17.0004''N$, $77^\circ 45'0.4680''E$), India in light of increasing concentrations of greywater contaminants after lunch hours. Greywater sampling was conducted in acid-washed glass vials, followed by immediate transport to the laboratory to preserve the samples below $4^\circ C$. Before the experiments, suspended solids and other impurities present in the greywater samples were filtered through Whatman nylon membrane filter paper (grade 1; pore size of $0.45\ \mu m$).

Pesticides levels in greywater samples depend on their existence and washing off from the vegetables and fruits. In the present study, the concentrations of CBZ and THX in raw greywater samples were only in trace quantities that are not inadequate to assess the biodegradation effects. Therefore, the collected greywater samples were used simply as matrix for spiking standard solutions of CBZ and THX for the degradation studies. After that, the use of three kinds of microbial cultures (i.e., (i) *A. versicolor* fungus, (ii) the bacterial consortium derived from kitchen waste sludge, and (iii) the augmented bacterial consortium with *A. versicolor*) was assessed towards the bioremediation of CBZ and THX. The

as-obtained experimental data were simulated with the aid of the first-order kinetic model to assess the rate constant values and half-life periods of pesticide degradation. A facile analytical method based on the LC-MS/MS technique was used to quantify the target pesticides in the greywater matrix. In this respect, a list of analytical parameters (e.g., accuracy, precision, calibration, limit of detection (LOD), and limit of quantification (LOQ)) were evaluated for quality assurance (QA) and quality control (QC) assured testing.

2.3. Bacterial and fungal culture conditions

Mixed cultures of microbes were isolated via the enrichment culture technique from the kitchen waste sludge collected at the Shriram Institute (Bangalore, Karnataka, India). The sludge sample was serially diluted and then streaked on nutrient agar media for culture cultivation. The streaked plate was then incubated at 37 °C in an incubator for 24 h. After 24 h, the agar plates were taken from the incubator, and the pure colony from the consortium was isolated via the streak plate method. Several visible colonies were observed to be grown in the petri dish. After the sub-culturing, pure colonies were obtained and preserved in test tubes containing nutrient agar slant at 4 °C until further usage. Identification of the colonies and morphological characteristics of the bacterial consortium are listed in Table S1. Relevant microscopic images are also shown in Figure S1.

A. versicolor (Strain no. MTCC 16858) was procured from IMTech (Chandigarh, Punjab, India). The pure culture was established in potato dextrose agar (PDA) medium at the microbiology laboratory of the Shriram Institute for Industrial Research (Bangalore, Karnataka, India). Fully-grown enzyme-secreting large flocs of fungi were disintegrated into fine fragments by sonication for 5 min under sterile conditions. The obtained biomass was transferred to a 300-mL conical flask at 28 °C. Each flask contains a 100-mL growth medium was incubated at 37 °C at 80 rpm for a week (Segura et al., 1993).

Equal volumes of pure fungus and bacterial consortium (50 mL each) were transferred into an Erlenmeyer flask containing soybean casein agar (SCA) medium at pH 7.3. The as-obtained mixed culture was incubated at 32 °C for 96 h (Rajpal et al., 2021). Consequently, the obtained augmented consortium (isolated mixed culture and fungus) was used as a source of inoculum for pesticide degradation.

2.4. Analytical reliability test for LC-MS/MS

Stock solutions of CBZ and THX pesticides were prepared at 1 ppm each (1000 µg/L) by adding 1 mg of each pesticide separately in 1000 mL methanol (SANTE, 2019). Secondary standard solutions for each pesticide were prepared at six different concentrations (i.e., 5, 10, 25, 50, 100, and 200 µg/L) by the serial dilution of the primary standard solution in DI. These standard solutions were stored in the refrigerator at 4 °C until further use. The suitability of the LC-MS/MS technique for the quantification of pesticides was assessed with the aid of basic QA procedures for DL, recovery, and precision, etc. For instance, 500 mL of greywater sample was spiked with 0.1 mL of the standard solutions of the pesticides (100 µg/L) and then QuEChERS EN sample extraction and d-SPE clean-up procedures were performed. Samples of the un-spiked greywater were also prepared similarly. These samples were analyzed using the LC-MS/MS technique in triplicate to estimate their average recoveries. The percentage recoveries were calculated using Eq. (1). The same procedure was replicated for all pesticide standards.

$$\% \text{Recovery} = \frac{SSR - USR}{SSC} \times 100, \quad (1)$$

where SSR, USR, and SSC denote the spiked sample results, un-spiked sample results, and spiked standard concentrations, respectively. To test the precision of the LC-MS/MS unit, 100 µg/L of the pesticides were analyzed repetitively for all the sample matrices ($n = 6$).

The reliability of the LC-MS/MS technique for the quantitation of the pesticide residues present in the greywater matrix was assessed through a series of quality assurance (QA/QC) analyses. The standard solution of pesticides in methanol at 10 µg/L and the extract of greywater samples were analyzed comparatively using LC-MS/MS. The resolutions of the analyte peaks from the nearest eluting peaks were also evaluated along with their retention times. They were used to calculate the LOD and LOQ. It is worth mentioning that the LOD is the minimum quantity of an analyte in a sample that can be consistently detected with a stated probability of $\geq 95\%$ (Shrivastava and Gupta, 2011). The LOD of the target pesticides were determined by replicate runs (3 times) of the analyte standards with the least concentration (Eq. (2)). Similarly, LOQ can be determined using Eq. (3) and corresponds to the least analyzable concentration of the analyte in the sample.

$$LOD = 3 * \frac{\alpha}{S} \quad (2)$$

$$LOQ = 10 * \frac{\alpha}{S} \quad (3)$$

where α and S are the standard deviation of a replicate measurement and slope of the calibration curve, respectively.

Table 1

Specification for greywater analysis by LC-MS/MS.

Order	Pesticide	Ionization mode	RT (min)	MM g/Mol	SRM1/SRM2 (m/z)	CE (Ev)	DP (V)	Accuracy %
1.	CBZ	Positive	9.59	191.187	292.0 160.0 132.0	5	82	100.37
2.	THX	Positive	7.15	291.71	291.9 210.9 131.9	7	56	100.2

*RT – Retention time; MM – Molar Mass; SRM – Selected reaction monitoring; CE – Capillary electrophoresis; DP – De-clustering potential.

2.5. Biodegradation experiments and pesticide analysis

Matrix spiking is a useful approach to assess the reliability of an analytical procedure when dealing with samples containing a low level of analytes. As explained above, collected greywater samples with trace quantities of target pesticide components were thus spiked with known quantities of their standard solutions. In detail, 500 mL of greywater sample was spiked with 100 µg/L of CBZ and THX each. The spiked greywater solution was then transferred to an autoclaved conical flask. An inoculation loop of the bioaugmented culture (diameter 5 mm) was aseptically transferred to the spiked greywater solution. The flask was covered with aluminum foil and placed in an incubator at 30 °C at an orbital shaking speed of 80 rpm. Similarly, spiked greywater samples were also incubated with fungus and the isolated consortium separately. Each experiment was conducted in duplicate for 240 h in order to achieve the complete degradation of pesticides. The experimental conditions were chosen by following the procedures described in earlier literature (Hai et al., 2012; Chin-Pampillo et al., 2015). A blank control solution was also prepared as a reference solution. The physicochemical characteristics of the greywater samples before and after treatment with the bacterial consortium, *A. versicolor*, and augmented consortium are summarized in Table S2. Note that the physicochemical parameters of greywater were only studied up to 96 h as their properties varied much less prominently over such period than the duration set for the whole removal test (e.g., 240 h: see below).

To analyze the biodegradation kinetics, the obtained data were evaluated using the first-order kinetic model as shown in Eq. (4) as follows (Bhandari et al., 2021).

$$\log C_t/C_0 = -kt, \quad (4)$$

where k (h^{-1}) is the first-order rate constant, $t^{1/2}$ is the half-life period of pesticides as given in Eq. (5) (Hamad, 2020), and C_0 and C_t are the initial and instantaneous concentrations of pesticides at any time “ t ”, respectively, and

$$t^{1/2} = \ln 2/k. \quad (5)$$

The concentrations of CBZ and THX were measured using the Agilent 6460 liquid chromatography (LC) equipped with a mass quadrupole-tandem mass spectrometer (MS) and Mass Hunter software (Agilent Technologies, triple Quad, version B.04.01). The specifications of the LC-MS/MS method used for the quantitative analysis of CBZ and THX are summarized in Table 1. An untargeted metabolomics-based approach was used for CBZ and THX analysis on the LC-MS/MS. Analysis was performed in positive ionization mode as the analyte composition was known. An Eclipse XDB-C18 column (50 mm × 2.1 mm; Agilent Technologies, Santa Clara, CA, USA) was employed as a stationary phase to perform separation of compounds. The dynamic mobile phase system consisted of Eluent A (5 mM aqueous ammonium formate solution (80%) + methanol (20%)) and eluent B (5 mM aqueous ammonium formate (10%) + methanol (90%)). The mobile phase gradients used to separate compounds were as follows (expressed as %B): 5%–100% for 10 min with 18 min held at 100% and 100%–5% for 1 min with 6 min held at 5%. The flow rate of the eluent system was fixed at 0.5 mL min^{−1} with the total analysis time for each run at 25 min. The sample injection volume was 5 µL. The fragmentation energy was set at 150 V, while the mass range for fragments was set between 50 and 1000 m/z at one spectra s^{−1}. Before each experiment, the instrument was calibrated with a maximum residual error of <0.2 ppm. The sample order was randomized using a random number generator, and all samples were analyzed in one continuous run to ensure ionization consistency (Zaibel et al., 2020).

A known volume of the greywater sample was placed in a separating funnel and extracted using methylene chloride at a neutral pH using the continuous liquid/liquid extraction (CLLE) methodology. This process was repeated thrice with methylene chloride (100 mL) in each extraction step. Subsequently, the methylene chloride layer was separated from the aqueous layer and dried over anhydrous sodium sulfate. The as-obtained greywater extract was subjected to a rotary evaporator to remove methylene chloride and then reconstituted with methanol. Lastly, the methanolic extract was filtered using a 0.22 µm polytetrafluoroethylene membrane filter (PTFE) to remove suspended impurities. The filtered extract was then analyzed using LC-MS/MS to determine the pesticide content using following formula (USEPA, 2007):

$$\text{Pesticide content (ppb)} = \left(\frac{\text{Area of sample}}{\text{Area of standard}} \right) \times (\text{standard concentration} \times \text{dilution factor}). \quad (6)$$

2.6. Tools applied for toxicity assessment

The EPRIP was developed to predict the pesticide toxicity in soil, surface water, and ground water (Chormare and Kumar, 2022). In the present study, the toxicity factor for target pesticides was estimated with respect to their leaching from soil and harmful effects on organism (e.g., algae, daphnia, and fish) present in ground water, using Eq. (7).

$$EPRIP = TU_p \times S_{rem} \times 1000, \quad (7)$$

where TU_p = pesticide concentration in greywater/end point (EC50; LC50) (mg/L); S_{rem} = removal score; EC50 = fifty percent effective concentration in mg L⁻¹ (algae); and LC50 = fifty percent lethal concentration in mg L⁻¹. EPRIP could be further classified by considering: (a) the high, medium, and low-toxicity rank given to a compound based on its LC50 for aquatic (fish acute toxicity) organisms and (b) the high, medium, or low removal efficiency of the compound observed during the usage of untreated greywater (Köck-Schulmeyer et al., 2013).

It is noted that direct runoff and erosion with soil particles are the major pathways responsible for the transportation of pesticides to surface water (Kookana et al., 2005). The transportation of pesticides to the surface water should be governed by a number of factors such as the rates of soil processes (e.g., erosion, irrigation, precipitation), $t_{1/2}$ and K_{oc} (Aravinna et al., 2017). However, the impact of erosion is higher than that of the runoff due to the reusability of greywater in areas where direct run off does not occur. In addition to $t_{1/2}$, K_{oc} is an important parameter related to the transportation of pesticides. K_{oc} is a measurement of bonding (inter-molecular) affinity of a compound to the soil than water. Hence, large K_{oc} is responsible for higher erosion of water than for direct run off. Therefore, the erosion plays a vital role in the mobility of pesticides with high K_{oc} values.

AFI could be determined using the recharge rate (q) for the assessment of the leaching potential of pesticides to the shallow water table at 3 m. The content of organic carbon and the density of the microbial population were considered to calculate AFI. Cumulative AFI was calculated to estimate the concentration of pesticides leached to the water table by using the following equation (Köck-Schulmeyer et al., 2013):

$$AFI = \exp\left(\frac{0.936D\theta_{FC}RF}{qt^{1/2}}\right), \quad (8)$$

where q = recharge rate; D = depth of soil profile to water table; θ_{FC} = volumetric moisture content of soil at field capacity; RF = retardation factor; and $t^{1/2}$ = environmental half-life of the pesticide.

$$RF = \left(1 + \frac{\rho K_{oc} f_{oc}}{\theta_{FC}}\right), \quad (9)$$

where ρ = bulk density of soil; K_{oc} = organic carbon partition coefficient; and f_{oc} = content of organic matter present in the soil.

The maximum expected concentration of each pesticide (C_{gw} : $\mu\text{g/L}$) was determined using the following equation:

$$C_{GW} = \frac{L_T \times AFI}{\theta_s} = \frac{(n \times r \times F_{AI}) \times AFI}{\theta_s}, \quad (10)$$

where n = frequency of application of pesticides; r = rate of application of pesticides; F_{AI} = fraction of active ingredients of the pesticides; L_T = total load of the pesticides on the catchment; θ_s = volume of voids per cubic meter of aquifer (porosity); and AFI = attenuation factor index. It should be noted that it was assumed that each pesticide that leached into the groundwater was uniformly distributed in a one-meter depth of the aquifer. The values of n , r , and L_T were taken from literature (Aravinna et al., 2017).

3. Results and discussion

3.1. Growth profile of the augmented consortium

Fig. 1 shows digital pictures of the growth of the bioaugmented culture of *A. versicolor* with the bacterial consortium in 240 h. Accordingly, the turbidity in the conical flasks increased steadily with time to reflect the growth of biomass in the sample. The increase in the cell-dry mass with time is shown in Fig. 2. It was observed that the initial lag phase for growth of the augmented consortium in composite broth medium was nearly 30 h, followed by the exponential growth phase for the next ~80 h. The stationary phase in the growth of the augmented consortium was then observed after 90 h accompanied by slight reduction in cell dry mass near 240 h. Hence, a period of 240 h was chosen to study bioremediation of CBZ and THX by the augmented consortium due to their complex chemical structures.

3.2. QA/QC analysis

The calibration plots for CBZ and THX were drawn across various concentrations (5–200 $\mu\text{g/L}$) as seen in Figure S2. The results showed good linearity and recoveries, such as 96.7% for CBZ and 84.9% for THX. Our analysis was comparable with the recovery values reported for DI-SPME (70%–120%) (Carneiro et al., 2013; Sante, 2015; Sapahin et al., 2019). The

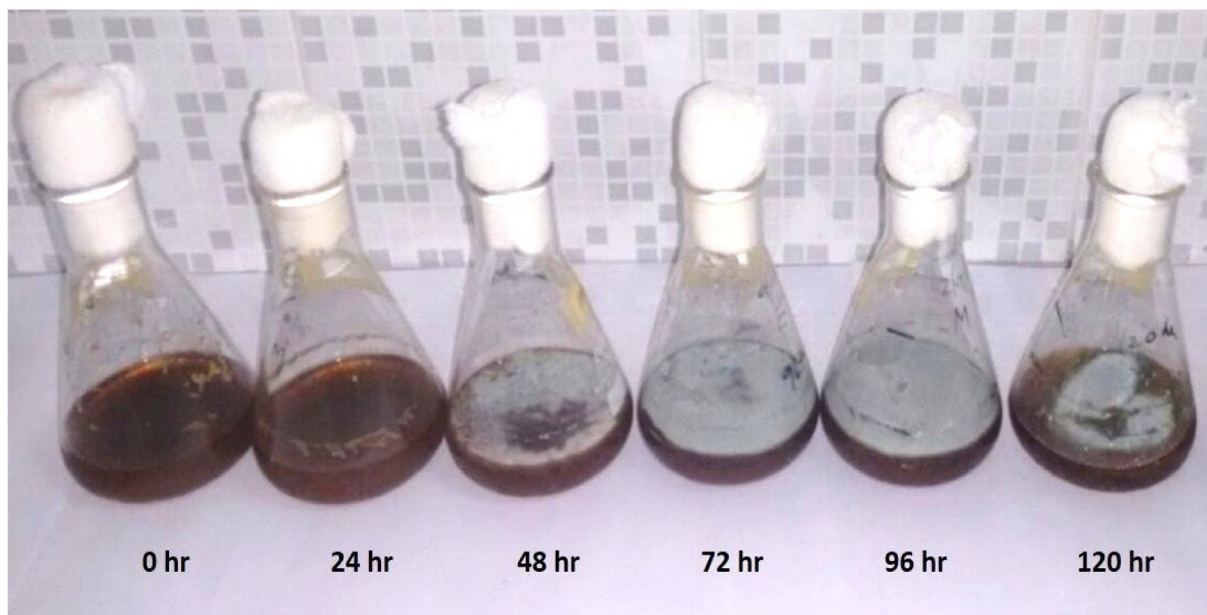


Fig. 1. Pictures of the growth of the augmented consortium with *A. Versicolor* at each 24 h interval in submerged media.

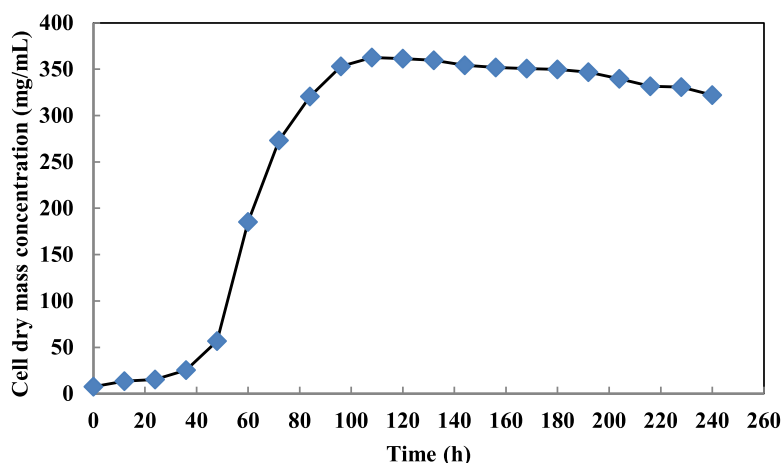


Fig. 2. Growth of the cell dry mass concentration of the augmented consortium as a function of time.

QuEChERS method and LC-MS coupled with the tandem mass spectrometry (MS/MS) technique were reliable, accurate, and suitable for the simultaneous determination of pesticide residues in greywater samples with good recoveries. The relative standard deviations (%RSD) in the quantification of CBZ and THX were found to be 0.69% and 0.84%, respectively (Table S3). Based on our analysis, the LOD values for CBZ and THX were calculated as 0.271 and 0.273 $\mu\text{g/L}$, respectively. The obtained LOD values were significantly lower than the lowest calibration level (5 $\mu\text{g/L}$) and the EU recommended maximum residue limits for the total concentration of the pesticides such as CBZ (10 $\mu\text{g/L}$) and THX (10 $\mu\text{g/L}$) (Union, 2012).

3.3. Pesticide degradation and kinetic analysis

The removal of CBZ and THX present in greywater samples was studied through the introduction of: (1) fungal culture, (2) isolated microbial consortium, and (3) augmented microbial consortium with fungus. The removal efficiencies for CBZ and THX were estimated relative to their initial concentrations in the spiked greywater samples. The profiles of the biodegradation of CBZ and THX on the three biomaterials are shown in Fig. 3. The biodegradation efficiencies for CBZ and THX obtained with the isolated bacterial consortium (Fig. 3a) were estimated for an incubation period of 240 h. The

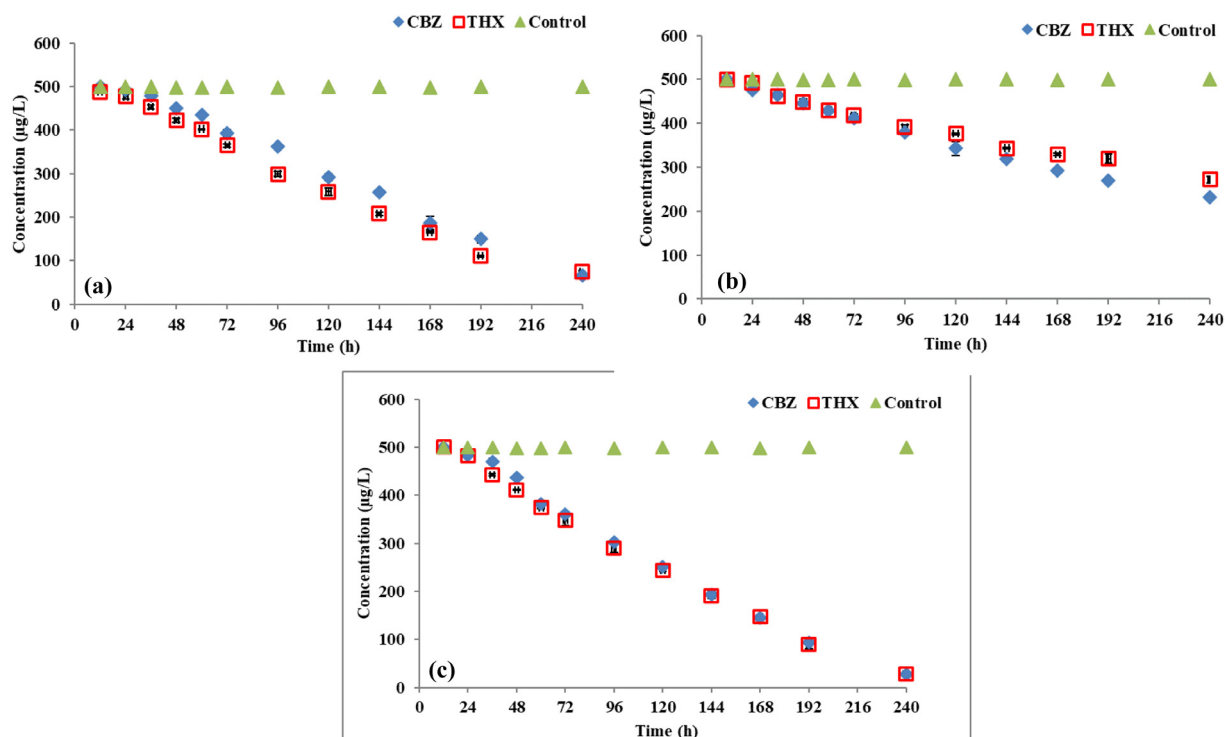


Fig. 3. Degradation profiles of pesticides with the tested cultures: (a) fungus, (b) bacterial consortium, and (c) augmented consortium.

removal percentages of CBZ and THX in spiked greywater samples by the isolated microbial consortium were 54.12% and 44.03%, respectively. The decrease in the concentrations implied that the bacteria consumed CBZ and THX as carbon and energy sources for its growth. However, the degrading efficiency of the bacterial consortium was lower than that of the fungal culture.

The *A. versicolor* fungal culture degraded 87.93% of CBZ and 84.80% of THX in 240 h. Biodegradation of CBZ and THX was facilitated by extracellular enzymes produced by the fungal culture (Rani et al., 2014). Such enzymes created therapeutic effects around their territory to trigger the degradation of pesticide molecules present in the greywater (Kellogg and Raja, 2017). The CBZ and THX adsorbed onto the fungus could also be degraded by releasing fungal metabolites. The CBZ removal efficiency was higher than the THX removal efficiency because of the enhanced release of extracellular enzymes from CBZ by the selected fungus (e.g., relative to THX). It is worth mentioning here that the culture was unacclimated to the mixture of CBZ and THX.

The bioaugmented consortium of bacteria and fungus exhibited different degradation trends for CBZ and THX from the fungal and bacterial consortium. On the 10th day, the concentrations of CBZ and THX were decreased by 94.4 and 93.6%, respectively, using the bioaugmented consortium. The effectiveness of combining fungi and bacteria could be further confirmed by comparing the removal rates of CBZ and THX by the three microbial cultures. Metabolic cooperation is essential for maintaining microbial consortia of multiple species to accomplish biological treatment of wastewater samples containing pesticides (Ijoma and Tekere, 2017). The appreciation of mutualism or commensalism relationships in the biofilms of mixed cultures would help to fabricate biofilms for the simultaneous biodegradation of recalcitrant pesticides. Therefore, it can be inferred that the augmented consortium implemented in this study showed the highest pesticide removal capacity, despite not being acclimated to the pesticide initially. From a practical point of view, the use of non-acclimated microbial cultures for pesticide degradation can be used as a beneficial option.

The identification of CBZ and THX in the un-spiked (or raw) and spiked greywater samples was performed for samples obtained before and after treatment using LC-MS/MS. In the un-spiked greywater, CBZ and THX peaks were observed at 6.9 min and 9.8 min, respectively. Upon spiking, the CBZ and THX peaks were shifted towards lower retention times due to the change in solution pH from 6.2 to 7.4. The treated spiked greywater also showed a change in retention time attributed to the change in the pH. The greywater samples with different cultures exhibited an intense peak at $m/z = 192.0$ along with daughter fragments at $m/z = 160.0$ and 132.0 for CBZ in positive multiple reaction monitoring (MRM) mode. Similarly, THX revealed an intense peak at $m/z = 291.9$ along with two daughter fragments at $m/z = 210.9$ and 131.9 .

The degradation kinetics of CBZ and THX in the greywater by the bioaugmented consortium of bacteria and fungus were simulated using the first-order reaction kinetic equation. Fig. 4 shows the linearly fitted degradation profiles of CBZ and THX by the tested microbial cultures.

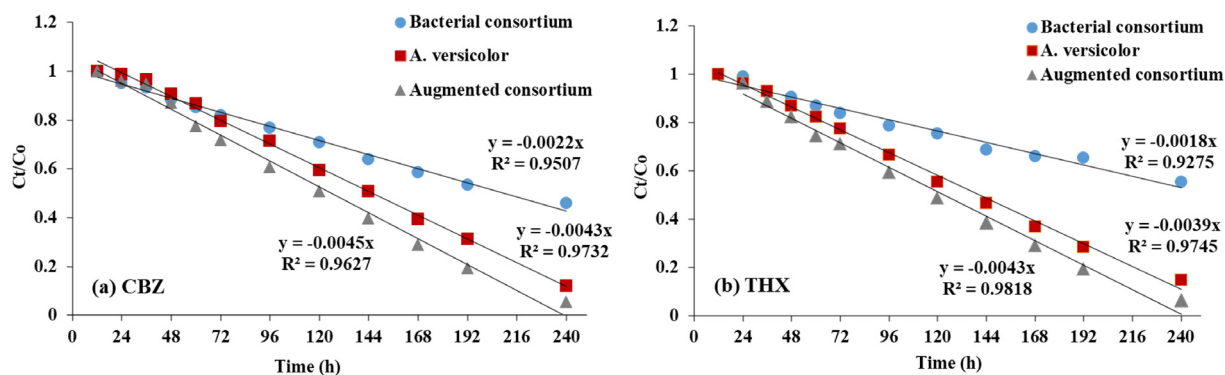


Fig. 4. First order kinetic equation for pesticide degradation by different cultures: (a) CBZ and (b) THX.

Table 2

Kinetic analysis of CBZ and THX degradation.

Order	Culture name	CBZ			TMX		
		K (h^{-1})	$T_{1/2}$ (h)	R^2	K (h^{-1})	$T_{1/2}$ (h)	R^2
1.	<i>A. versicolor</i>	5.83	55.25	0.97	5.11	76.87	0.96
2.	Bacterial consortium	2.13	184.80	0.96	1.63	156	0.95
3.	Augmented consortium	8.06	48.00	0.99	7.94	62.45	0.98

K-first order rate constant; $T_{1/2}$ -half-life; and R^2 -Coefficient of determination.

Table 3

Comparison of biodegradation rates of CBZ and THX with other consortiums.

Order	Consortium of microbes	Pesticide degradation	References
1.	<i>Acinetobacter</i> sp., <i>Enterobacter</i> sp. and <i>Bacillus</i> sp	THX 91.34%	Hegde et al. (2017)
2.	Consortia of <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Exiguobacterium</i> , <i>Microbacterium</i> , <i>Achromobacterium</i> , <i>Stenotrophomonas</i>	CBZ 77.5%	Kanjilal et al. (2018)
3.	Consortia of <i>Bacillus</i> sp. <i>Paracoccus</i> sp. <i>Flavobacterium</i> sp. <i>Pseudomonas</i> sp	CBZ 87.25%	Singh et al. (2016)
4.	<i>Brevibacillus borstelensis</i> and <i>Streptomyces albogriseolus</i>	CBZ 88%	Arya and Sharma (2016)
5.	<i>Bacillus subtilis</i> GB03, <i>Bacillus subtilis</i> FZB24, <i>Bacillus amyloliquefaciens</i> IN937a and <i>Bacillus pumilus</i> SE34	THX 22%	Bhattacharjee et al. (2020)
6.	Isolated consortium augmented with <i>Aspergillus versicolor</i>	CBZ 94.29% THX 93.41%	Present study

The results of the kinetic analysis indicated a high coefficient of determination ($R^2 = >0.9$) for the removal of the target pesticides (Table 2). This result suggested that the first order kinetic equation could be successfully applied to simulate the removal of the target pesticides by the tested cultures. This result also indicated that the kinetic biodegradation rate of the CBZ and THX pesticides by microbial cultures was heavily dependent on the initial pesticide concentrations in the greywater matrix. The biodegradation half-life periods ($t_{1/2}$) of CBZ and THX by *A. versicolor* were found to be 55.25 and 76.87 h, respectively. Therefore, *A. versicolor* showed a higher potency towards CBZ than to THX. Similarly, their $t_{1/2}$ values for bacterial consortium were 184.8 and 156 h, respectively. On the other hand, the augmented consortium revealed relatively low $t_{1/2}$ values for CBZ and THX of 48 and 62.45 h, respectively. This result indicated that the augmentation of the bacterial consortium with *A. versicolor* was an effective practical approach to boost its performance in bioremediation of wastewater. The augmented consortium also demonstrated the highest values of the kinetic rate constant of CBZ (8.06 h^{-1}) and THX (7.94 h^{-1}) in greywater.

The biodegradation rate by the bioaugmented consortium was compared with those of other reported consortiums of strains as shown in Table 3. A half-life of 76.3 h was observed for the biodegradation of CBZ with a mixed bacterial culture (Njalam'mano et al., 2020), while that for THX was 96 h using a consortium of bacterial strains (Rodríguez-Castillo et al.,

Table 4
EPRIP scores and corresponding toxicity assessment (Padovani et al., 2004).

EPRIP judgement value	EPRIP judgement	Removal (%)	Removal rate (S_{rem})	EPRIP score	EPRIP score for CBZ	EPRIP score for THX
1	Negligible	75–100	0.2	02–16.0	42.1 this study	6.4 this study
2	Small	50–75	0.4	17–81		
3	Present	25–50	0.6	82–256		
4	Large	0–25	0.8	257–400		
5	Very large	<0	1	>400		

2019). In this study, the half-lives of the biodegradation of CBZ and TMX by bioaugmented microbial consortium were estimated to be 84–92 h and 82–92 h, respectively. Our results were in good agreement with those of an earlier study for the biodegradation of isoproturon in agricultural soil by a *Sphingomonas* sp. strain and a microbial consortium (Li et al., 2017). The results of the present study also indicated that the bioaugmented consortium was more effective in causing biodegradation of xenobiotic compounds than the homogeneous consortium of bacteria and fungus were individually. Its biodiversity supports environmental survival while expanding the number of catabolic pathways available for contaminant biodegradation (Blasche et al., 2017). Microbial degradation was used more frequently because pesticides were used primarily as microbial nutrients, while being ultimately decomposed into some small non-hazardous molecules, such as CO_2 and H_2O (Rajpal et al., 2020).

The process of microbial degradation was prolonged when accompanied with structural modifications. Among all of the options, the augmented microbial consortium was the most effective culture at achieving pesticide degradation (e.g., relative to the bacterial consortium and the pure fungal strain). Therefore, it was suspected that the augmented microbial consortium contained different types of microorganisms, such as bacteria, archaea, and fungi. A larger microbial population could facilitate complete biodegradation of the pesticides into carbon dioxide and water to provide energy to the microbes for their metabolism. Rapid microbial growth of the augmented consortium in the greywater samples contaminated with pesticides indicated that both CBZ and THX could be consumed as a carbon source and for the associated bioremediation. Moreover, the relatively quicker degradation of CBZ than THX could suggest the preferential consumption of the former by the specific enzymes produced by microbes present in the augmented consortium.

3.4. Toxicity assessment

The recommended acute toxicity indicators (i.e., LC_{50} and EC_{50}) were used to assess the hazardous effect of the target pesticides for aquatic organisms. In case of soil leached CBZ, the values of LC_{50} for earthworms and EC_{50} for aquatic organisms were found to be 5.8 and 0.15 mg/L, respectively (PPDB, 2022). The reference parameters for the estimation of EPRIP values were the EU's permissible limit of pesticides in drinking water (0.1 mg/L), LC_{50} values for fish and daphnia (low = >100; medium = 0.1–100; and high = <0.1), and EC_{50} for algae (low = >10, medium = 0.01–10, and high = <0.01) (Padovani et al., 2004). The recommended toxicity indicators were moderate to fall in the range of permissible values. As shown in Table 4, the EPRIP index calculations and relevant analysis indicated that CBZ (42.1) could pose more toxicity to the aquatic environment than THX (6.4). This difference could be accounted for by the combined effects of various factors (e.g., the environmental fate, transport pathway of the active ingredients, and toxicological effects of the active ingredients of CBZ). After an incubation period of 240 h, the concentrations of CBZ and THX in the spiked greywater samples were reduced to 28 $\mu\text{g/L}$ and 32 $\mu\text{g/L}$, respectively (e.g., by more than 93% of the initial concentration of 500 $\mu\text{g/L}$), which were lower than their maximum allowable concentration of 0.1 mg/L (WHO, 1993).

Black soil is the major soil type in Bangalore city in India with a sandy clay loam texture on the surface and subsurface soils. The values of ρ and θ_s for soil are taken from the literature and shown in Table S4. The recharge rate for the soil profile was estimated to be 20 to 50 mm h^{-1} (Rao et al., 2018). The AFI values estimated for CBZ and THX were 1003.4 and 1001.2, respectively. The lower AFI for THX indicated higher vulnerability of the underlying groundwater. The higher recharge rate in groundwater for the contaminant implied that the concentration of the contaminant moving through the unsaturated zone was diluted more efficiently. Thus, the impact on water quality was mild. Contaminants with higher solubility in water or polarity are also rapidly transported through the unsaturated zone. The two processes usually do not have an impact on vulnerability at the same magnitude. In addition, the intensity and duration of rainfall events also affects the nature and magnitude of contaminates in soil. The AFI, r , and F_{AI} values are essential parameters that affect the C_{GW} of a pesticide (all other parameters are constant for both pesticides). The effect of AFI on C_{GW} is more significant than that in pesticide application data (r and FAI) (Padovani et al., 2004). Therefore, AFI is the critical parameter that can affect C_{GW} . The residue level of pesticides as estimated at a 0.3 m water table was higher for CBZ (936 ng/L) than for THX (167 ng/L). The results of this study indicated that CBZ was more mobile than THX. However, as these predictions were made based on K_{oc} and $t_{1/2}$ values in the literature, it can contribute to the uncertainty of the predictions. It is further anticipated that the values of AFI and EPRIP estimated in this study would tend to vary with environmental conditions, such as pH and soil microbiological activity.

4. Conclusions

In the present study, a naturally derived bacterial consortium was augmented with a fungus (*A. versicolor*) to enhance their performance for the biodegradation of carbendazim and thiamethoxam pesticides present in greywater. The results showed that the biodegradation efficiencies of carbendazim and thiamethoxam were estimated to be 94.29% and 92.41%, respectively, using the augmented consortium. It was observed that the augmented consortium outperformed its constituting cultures, i.e., the naturally obtained consortium and individual fungal culture, due to the therapeutic effects of its extracellular enzymes. Furthermore, the carbendazim removal efficiency was higher than that of thiamethoxam and that could likely be attributed to the higher affinity of the enzyme towards carbendazim. The mobility of carbendazim and thiamethoxam in water was predicted to assess their toxic effects on the environment. The values of the EPRIP index for carbendazim and thiamethoxam were estimated as 42.1 and 6.4, respectively. Accordingly, it is proposed that carbendazim could pose threat to the environment more significantly than thiamethoxam. The values of AFI and C_{GW} for carbendazim were calculated as 1003.4 and 936 ng/L, respectively, while their counterparts for thiamethoxam were 1001.2 and 167 ng/L, respectively. Based on the AFI values, it could be inferred that carbendazim is a more mobile pesticide for penetrating from soil into groundwater than thiamethoxam. The risk of migration of carbendazim in humans is low, although it can be high in aquatic animals (e.g., daphnia, fish, and macrophytes). The results of the toxicity indicators showed that groundwater contamination by pesticides rely on the multitude of variables (e.g., hydrological, pesticides, soil properties, and climatic conditions). The overall results of this study suggest that the cultures augmented with *A. versicolor* can be used as a promising tool to enhance the removal efficiency of the toxic pesticides persisting in the biosystems. It is expected that the results of the present study will provide helpful information on the utility of mixed cultures in wastewater treatment.

CRedit authorship contribution statement

Nikita Rajpal: Investigation, Methodology, Data curation, Formal analysis, Writing – review & editing. **Swati Verma:** Investigation, Methodology, Data curation, Formal analysis, Writing – review & editing. **Navneet Kumar:** Conceptualization, Formal analysis, Writing – review & editing. **Jechan Lee:** Conceptualization, Formal analysis, Writing – review & editing. **Ki-Hyun Kim:** Conceptualization, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Jatinder K. Ratan:** Conceptualization, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Neetu Divya:** Conceptualization, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.eti.2023.103087>.

References

- Abdel-Razek, M.A., Abozeid, A.M., Eltholth, M.M., Abouelenien, F.A., El-Midany, S.A., Moustafa, N.Y., Mohamed, R.A., 2019. Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria. *Slov. Vet.* 56, 61–73.
- Aravinna, P., Priyantha, N., Pitawala, A., Yatigammana, S.K., 2017. Use pattern of pesticides and their predicted mobility into shallow groundwater and surface water bodies of paddy lands in smahaweli river basin in Sri Lanka. *J. Environ. Sci. Health, Part B* 52, 37–47.
- Arya, R., Sharma, A.K., 2016. Bioremediation of carbendazim a benzimidazole fungicide using *brevibacillus borstelensis* and *streptomyces albogriseolus* together. *Curr. Pharmaceut. Biotechnol.* 17, 185–189.
- Bhandari, G., Bhatt, P., Gangola, S., Srivastava, A., Sharma, A., 2021. Degradation mechanism and kinetics of carbendazim using *achromobacter* sp. strain GB61. *Bioremed. J.* 1–17.
- Bhattacharjee, A.K., Shukla, P.K., Dikshit, A., 2020. Microbial Biotransformation of neonicotinoid insecticides in soil—A review. *Int. J. Curr. Microbiol. App. Sci.* 9, 3255–3277.

- Blasche, S., Kim, Y., Oliveira, A.P., Patil, K.R., 2017. Model microbial communities for ecosystems biology. *Curr. Opin. Syst. Biol.* 6, 51–57.
- Bonnechère, A., Hanot, V., Bragard, C., Bedoret, T., Loco, J.Van., 2012. Effect of household and industrial processing on the levels of pesticide residues and degradation products in melons. *Food Additives Contaminants: Part A* 29, 1058–1066.
- Carazo-Rojas, E., Pérez-Rojas, G., Pérez-Villanueva, M., Chinchilla-Soto, C., Chin-Pampillo, J.S., Aguilar-Mora, P., Alpizar-Marín, M., Masís-Mora, M., Rodríguez-Rodríguez, C.E., Vryzas, Z., 2018. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. *Environ. Pollut.* 241, 800–809.
- Carneiro, R.P., Oliveira, F.A., Madureira, F.D., Silva, G., de Souza, W.R., Lopes, R.P., 2013. Development and method validation for determination of 128 pesticides in bananas by modified QuEChERS and UHPLC–MS/MS analysis. *Food Control* 33, 413–423.
- Castro-Gutiérrez, V., Masís-Mora, M., Carazo-Rojas, E., Mora-López, M., Rodríguez-Rodríguez, C.E., 2019. Fungal and bacterial co-bioaugmentation of a pesticide-degrading biomixture: pesticide removal and community structure variations during different treatments. *Water Air Soil Pollut.* 230, 1–13.
- Chin-Pampillo, J.S., Ruiz-Hidalgo, K., Masís-Mora, M., Carazo-Rojas, E., Rodríguez-Rodríguez, C.E., 2015. Adaptation of biomixtures for carbofuran degradation in on-farm biopurification systems in tropical regions. *Environ. Sci. Pollut. Res.* 22, 9839–9848.
- Chormare, R., Kumar, M.A., 2022. Environmental health and risk assessment metrics with special mention to biotransfer bioaccumulation and biomagnification of environmental pollutants. *Chemosphere* 302, 134836.
- de Souza, R.M., Seibert, D., Quesada, H.B., Bassetti, F.deJesus, Fagundes-Klen, M.R., Bergamasco, R., 2020. Occurrence impacts and general aspects of pesticides in surface water: a review. *Process Safety Environ. Protect.* 135, 22–37.
- Fang, H., Zhang, H., Han, L., Mei, J., Ge, Q., Long, Z., Yu, Y., 2018. Exploring bacterial communities and biodegradation genes in activated sludge from pesticide wastewater treatment plants via metagenomic analysis. *Environ. Pollut.* 243, 1206–1216.
- Hai, F.I., Modin, O., Yamamoto, K., Fukushima, K., Nakajima, F., Nghiem, L.D., 2012. Pesticide removal by a mixed culture of bacteria and white-rot fungi. *J. Taiwan Inst. Chem. Eng.* 43, 459–462.
- Hamad, M.T.M.H., 2020. Biodegradation of diazinon by fungal strain *aspergillus niger* MK640786 using response surface methodology. *Environ. Technol. Innov.* 18, 100691.
- Hassan, A., Periathamby, A., Ahmed, A., Innocent, O., Hamid, F.S., 2020. Effective bioremediation of heavy metal-contaminated landfill soil through bioaugmentation using consortia of fungi. *J. Soils Sediments* 20, 66–80.
- Hegde, D.R., Manoharan, T., Sridar, R., 2017. Identification and characterization of bacterial isolates and their role in the degradation of neonicotinoid insecticide thiamethoxam. *J. Pure Appl. Microbio.* 11, 393–400.
- Ijoma, G., Tekere, M., 2017. Potential microbial applications of co-cultures involving ligninolytic fungi in the bioremediation of recalcitrant xenobiotic compounds. *Int. J. Environ. Sci. Technol.* 14, 1787–1806.
- Kanjilal, T., Panda, J., Datta, S., 2018. Assessing *brevibacillus* sp C17: An indigenous isolated bacterium as bioremediator for agrochemical effluent containing toxic carbendazim. *J. Water Process Eng.* 23, 174–185.
- Kellogg, J.J., Raja, H.A., 2017. Endolichenic fungi: a new source of rich bioactive secondary metabolites on the horizon. *Phytochem. Rev.* 16, 271–293.
- Kim, M.-K., Zoh, K.-D., 2016. Occurrence and removals of micropollutants in water environment. *Environ. Eng. Res.* 21, 319–332.
- Köck-Schulmeyer, M., Villagrana, M., de Alda, M.L., Céspedes-Sánchez, R., Ventura, F., Barceló, D., 2013. Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. *Sci. Total Environ.* 458, 466–476.
- Kookana, R.S., Correll, R.L., Miller, R.B., 2005. Pesticide impact rating index—a pesticide risk indicator for water quality. *Water, Air, Soil Pollut. Focus* 5, 45–65.
- Li, R., Dörfler, U., Munch, J.C., Schroll, R., 2017. Enhanced degradation of isoproturon in an agricultural soil by a *sphingomonas* sp strain and a microbial consortium. *Chemosphere* 168, 1169–1176.
- Li, H., Qiu, Y., Yao, T., Ma, Y., Zhang, H., Yang, X., Li, C., 2020. Evaluation of seven chemical pesticides by mixed microbial culture (PCS-1): Degradation ability microbial community, and medicago sativa phytotoxicity. *J. Hazardous Mater.* 389, 121834.
- Liu, N., Pan, X., Yang, Q., Ji, M., Zhang, Z., 2018. The dissipation of thiamethoxam and its main metabolite clothianidin during strawberry growth and jam-making process. *Sci. Rep.* 8 (15242).
- Maqbool, Z., Hussain, S., Imran, M., Mahmood, F., Shahzad, T., Ahmed, Z., Azeem, F., Muzammil, S., 2016. Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. *Environ. Sci. Pollut. Res.* 23, 16904–16925.
- Medaura, M.C., Guivernau, M., Moreno-Ventas, X., Prenafeta-Boldú, F.X., Viñas, M., 2021. Bioaugmentation of native fungi, an efficient strategy for the bioremediation of an aged industrially polluted soil with heavy hydrocarbons. *Front. Microbiol.* 12.
- Nie, J., Sun, Y., Zhou, Y., Kumar, M., Usman, M., Li, J., Shao, J., Wang, L., Tsang, D.C., 2020. Bioremediation of water containing pesticides by microalgae: mechanisms methods, and prospects for future research. *Sci. Total Environ.* 707, 136080.
- Njalam'mano, J.B., Chirwa, E.M., Seabi, R.L., 2020. In vitro study of butyric acid deodorization potential by indigenous constructed bacterial consortia and pure cultures from pit latrine fecal sludge. *Sustainability* 12.
- Noman, E.A., Al-Gheethi, A.A.S., Talib, B.A., Mohamed, R.M.S.R., Nagao, H., Kassim, A.H.M., 2019. Xenobiotic organic compounds in greywater and environmental health impacts. In: *Management of Greywater in Developing Countries*. Springer, pp. 89–108.
- Padovani, L., Trevisan, M., Capri, E., 2004. A calculation procedure to assess potential environmental risk of pesticides at the farm level. *Ecol. Indic.* 4, 111–123.
- PPDB, 2022. <http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/116.htm>.
- Rajpal, N., Divya, N., Sharma, S., Balasubramani, K., Ratan, J.K., 2020. Treatment of greywater using consortium of *micrococcus luteus* *rhodococcus equi* and *aspergillus niger*. *J. Indian Chem. Soc.* 97, 478–482.
- Rajpal, N., Ratan, J.K., Divya, N., Hebbani, A.V., 2021. Bioremediation of greywater using a novel bacterial–fungal consortium: optimization and validation of the operating parameters in vitro. *Environ. Technol.* 1–13.
- Rani, B., Kumar, V., Singh, J., Bisht, S., Teotia, P., Sharma, S., Kela, R., 2014. Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. *Braz. J. Microbiol.* 45, 1055–1063.
- Rao, D.A., Naik, P.K., Jain, S.K., Kumar, K.V., Rao, E.D., 2018. Assessment of vulnerability zones for ground water pollution using GIS-DRASTIC-EC model: A field-based approach. *J. Earth Syst. Sci.* 127, 1–24.
- Rodríguez, C., Sánchez, R., Rebollo, N., Schneider, N., Serrano, J., Leiva, E., 2021. Life cycle assessment of greywater treatment systems for water-reuse management in rural areas. *Sci. Total Environ.* 795, 148687.
- Rodríguez-Castillo, G., Molina-Rodríguez, M., Cambronero-Heinrichs, J.C., Quirós-Fournier, J.P., Lizano-Fallas, V., Jiménez-Rojas, C., Masís-Mora, M., Castro-Gutiérrez, V., Mata-Araya, I., Rodríguez-Rodríguez, C.E., 2019. Simultaneous removal of neonicotinoid insecticides by a microbial degrading consortium: detoxification at reactor scale. *Chemosphere* 235, 1097–1106.
- Sante, E., 2015. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. European Commission, pp. 1–46.
- SANTE, 2019. https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf.
- Sapahin, H.A., Makahleh, A., Saad, B., 2019. Determination of organophosphorus pesticide residues in vegetables using solid phase micro-extraction coupled with gas chromatography–flame photometric detector. *Arab. J. Chem.* 12, 1934–1944.

- Segura, F., Durany, G., Lozano, R., Huguet, V., 1993. Detoxification pretreatment of black liquor derived from non-wood feedstock with white-rot fungi. *Environ. Technol.* 14, 681–687.
- Shaikh, I.N., Ahammed, M.M., 2020. Quantity and quality characteristics of greywater: a review. *J. Environ. Manag.* 261, 110266.
- Shrivastava, A., Gupta, V.B., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles Young Sci.* 2, 21–25.
- Singh, S., Singh, N., Kumar, V., Datta, S., Wani, A.B., Singh, D., Singh, K., Singh, J., 2016. Toxicity. Monitoring and Biodegradation of the Fungicide Carbendazim. *Environmental Chemistry Letters* 14, 317–329.
- Union, E., 2012. Maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending council directive 91/414/EEC.
- USEPA, 2007. Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS. EPA method 1699.
- WHO, 1993. https://incchem.org/documents/hsg/hsg/hsg82_e.htm.
- Zaibel, I., Dagan, G., Arnon, S., Schwartzburd, F., Britzi, M., Snyder, S.A., Zilberg, D., 2020. Tertiary-treated wastewater as a potential water source for sustainable aquaculture: A laboratory-scale experiment with cyprinus carpio. *Aquaculture* 522, 735161.
- Zhang, Y., Xu, Z., Chen, Z., Wang, G., 2020. Simultaneous degradation of triazophos methamidophos and carbofuran pesticides in wastewater using an enterobacter bacterial bioreactor and analysis of toxicity and biosafety. *Chemosphere* 261, 128054.