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Aim and scope

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Comparison of Antibiotic Resistance in the influent and Effluent of Two Wastewater Treatment Plants

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ABSTRACT

The antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are considered new classes of water contaminants due to their potential adverse effects on aquatic ecosystems and human health. This paper describes the susceptibility evaluation of Escherichia coli, isolated from the influent and effluent of the biological reactors in two wastewater treatment plants (WWTP1 and WWTP2), and the evaluation of the disinfection efficiency of the UV radiation system in WWTP2.

The exposure to different families of antibiotics was evaluated, namely, macrolides (erythromycin, azithromycin, clarithromycin), quinolones (ofloxacin, ciprofloxacin), nitroimidazoles (metronidazole), sulfanilamides (sulfamethoxazole) and trimethoprim, the latter is used in combination with sulfamethoxazole due to its synergistic effect.

The results of the antimicrobial susceptibility tests, using disc diffusion (Kirby–Bauer) method, showed the occurrence of E. coli strains resistance. The analysis performed indicated an overall resistance (considering both influents and effluents) in WWTP1 and WWTP2, of respectively: 33.3% and 37.5% to erythromycin; 0.0% and 4.0% to azithromycin; 25.0% and 29.2% to clarithromycin; 12.5% and 4.2% to ofloxacin; 16.7% and 4.2% to ciprofloxacin; 29.2% and 12.5% to trimethoprim; 41.7% and 12.5% to sulfamethoxazole. A variability of resistance was observed along the studied period, from WWTP1 and WWTP2, and from influent to effluent.

Disinfection by UV demonstrated good performance, achieving in some samples 100% removal of E. coli that has growth in TBX agar. However, a proper supervision is needed in order to achieve the allowed limits concerning the number of bacteria.

The results from this work contribute to a better awareness of ARB dissemination from wastewater treatment plants to the aquatic environment.

Keywords: domestic wastewater treatment; antibiotics; Escherichia coli; antibiotic-resistance bacteria; UV disinfection

1. INTRODUCTION

Antibiotics play a crucial role in the treatment of numerous types of infectious diseases caused by pathogenic microbes. According to the European Centre for Disease Prevention and Control (ECDC), in 2017, the average utilization of antibiotics in the European Union was 22 defined daily doses (DDD) per 1000 habitants and ranged from 10 DDD in Netherlands to1 34 DDD in Cyprus [1]. Due to the high and continuous consumption of antibiotics they have been found in many aquatic systems, including in surface waters [2] and wastewaters [3]. Such occurrence has been accompanied by the proliferation of antibiotic resistance genes (ARG) and the appearance of resistant strains in the environment due to the increased selective pressure in bacterial population [4]. Today, the emergence and spread of antibiotic resistant bacteria (ARB) is one of the greatest threats to public health, seeing that this compromises the effectiveness of antimicrobial therapy, leading to more infections and causing higher morbidity and mortality [5,6].

The acquisition of genes that encode the resistance to antibiotics occur mainly via transfer of ARG between bacterial cells through plasmids and other mobile genetic elements, such as transposons or integrons [5]. Apart from horizontal gene transfer (HGT), the resistance of microbes to antibiotics may also arise through mutation [4,7]. Although the antibiotic resistance is an inevitable evolutionary consequence due to the natural selection process, the rate at which these processes occur and the number of resistant strains have greatly increased over the past years due to the selective pressure derived from anthropogenic activities [4].

Wastewater treatment plants (WWTPs) are important storage and emission sources of antibiotics and ARGs to the environment. Although WWTPs are useful for removing antibiotics and ARGs, they are not specifically designed for this task [8]. The effluents from municipal wastewater treatment plants (WWTPs) have been identified as the most significant anthropogenic sources for the dissemination of antibiotic residues, ARB and ARG, into the environment [7]. Since the antibiotics consumed are not completely metabolized they are excreted in urine and faeces, not only as metabolites but also as parent compounds into the wastewater [9,10]. Among the multiple pathways of antibiotic resistance dissemination, hospital effluents discharged into WWTPs represent a relevant source of ARG and ARB. Studies have demonstrated that hospital effluents contain higher prevalence of ARG, like blaTEM and vanA genes [11,12], higher concentrations of antibiotic residues [13], and higher loads of ARB than municipal effluents [14]. The high resistance rates of Enterococcus spp. to the antibiotics vancomycin, erythromycin and ciprofloxacin in Portugal were found in cities with a high number of hospital units [15]. The same phenomena has been observed in several countries [16,17]. Thus, thereis an increasing concern about the environmental risk posed by the wastewater discharge on receivers, namely due to the dissemination of antimicrobial resistance.

Conventional WWTPs assemble three sequential steps, preliminary, primary and secondary treatments, which have been designed to remove bulk solids, sand, floating fat, grease and biodegradable compounds from wastewater [18]. Considering that WWTPs have a limited capacity of removing micropollutants, such as antibiotics, these components end up in the aquatic environment creating selective pressure for the proliferation of ARB and ARGs in aquatic ecosystems [18–21]. In fact, WWTPs have been recognized as reservoirs for the spread of antibiotic resistance [22]. The microbial communities present in the activated sludge process, one of the most used secondary treatments, are highly concentrated. The presence in the biological reactor inflow of high nutrient content, under subinhibitory concentrations of antibiotics, fungicides and metals, create a favorable environment for the spread of ARGs [23]. The proximity between bacterial cells promotes genetic exchange through HGT of ARB, and the high nutrient content create the ideal conditions for survival and growth of ARB [7,20].

To minimize the risk of spreading microorganisms [3] disinfection processes have been implemented in some WWTPs before the discharge to receiving waters. Chlorine, ultraviolet (UV) radiation and ozonation are the most common disinfection technologies currently applied in WWTPs worldwide [18,24,25]

Due to the low cost of chlorine, it is the most common method of chemical disinfection. This method demands a high quality treated water, low levels of suspended solids, organic matter and ammoniacal nitrogen in order to guarantee its efficiency and reduce the formation of oxidation by-products, such as organochlorinated compounds that are toxic and carcinogenic [25].

Both UV and ozone are well suited disinfection agents concerning the objective of degradation and deactivation of ARGs. UV radiation causes photochemical damage to RNA and DNA, preventing the reproduction of cells [25]. It is directly absorbed by pyrimidine and purine nucleobases causing DNA mutations which then impairs the synthesis of critical proteins and DNA replication. As a consequence, in the absence of repair mechanisms, there will be cell death and inactivation of ARGs [10,26,27]. Ozonation works on a different principle, ozone can penetrate the bacterial cells, breaks the cell walls, and also promotes multiple oxidation reactions of cellular constituents damaging cell structures and DNA [10,27].

In general, these disinfection processes contribute to the effective decrease of microbial loads, but it has been demonstrated that they might have potential to act selectively over some bacterial groups. Under the stressful conditions imposed by the disinfection processes some of the bacteria species will die, whilst others will enter a dormant state recovering when the stressors are removed [18]. In this way, disinfection treatment may compromise the bacterial diversity, which will contribute to the enrichment of ubiquitous bacteria associated with acquirement of ARGs [28]. On the other hand, even if the disinfection process has shown an effective reduction of ARB, intact remnants of ARGs may prevail and confer antibiotic resistance to bacterial populations [26,29]. Thus, the disinfection process should be capable of deactivating ARGs and inactivating the corresponding ARB. There is still much to learn about the efficiency of tertiary treatment technologies (disinfection processes) for removal of ARB. In this context, the aim of the present work was to study the occurrence of antibiotic resistance of Escherichia coli isolated from the inflow and outflow of the biological reactors (secondary treatment) from two typical municipal WWTPs in the North of Portugal. Furthermore, the efficiency of UV radiation to inactive microorganisms was also evaluated. The antibiotics used in this study were selected by taking into account their presence and persistence in the environment [21,30]. Sulfonamides, fluoroquinolones and macrolides are the most persistent antibiotic families; tetracyclines are less mobile, but can persist for relatively long periods in the absence of sunlight; and aminoglycosides and β-lactam antibiotics show less persistence [21]. In previous studies in WWTP effluents, sulfamethoxazole and quinolones (in particular, ciprofloxacin and ofloxacin) were the antibiotics present at higher concentrations [3,30].

2. MATERIALS AND METHODS

2.1. Wastewater treatment plants

Sampling campaigns were performed in two urban WWTPs, which receive only domestic wastewater.

The WWTP1 was designed for 25,000 population equivalent and an average flowrate of 14,000 m3/d. This treatment plant has preliminary treatment (screening and removal of grit, as well as oil and grease), primary treatment (sedimentation) and secondary treatment carried out into a biological reactor (activated sludge in extended aeration regime).

The WWTP2 was designed for 80,000 population equivalent and an average flow rate of 7,000 m3/d. It has preliminary treatment (screening and grit removal), primary treatment (sedimentation), secondary treatment performed into a biological reactor (activated sludge in extended aeration regime) and tertiary treatment (micro screening and disinfection by ultraviolet irradiation), according to the discharge license this last step is only mandatory during the Summer.

2.2. Sampling procedure and wastewater characterization

A total of 12 sampling campaigns were performed in two urban WWTPs (WWTP1 and WWTP2) from March to June (2015) in weekdays. The samples were collected by dipping of a sterile 1 L bottle in the upstream and downstream flow of the biological reactor in both WWTPs under study, and after the disinfection step of WWTP2. Their transportation to the laboratory (30 min) was made under controlled temperature conditions, and they were immediately processed. Manipulation of the samples was made under aseptic conditions.

The sample collection inside the biological reactors was performed by dipping of a vessel. As they are continuous stirred tank reactors, the composition inside the reactors is the same as in its outlet. The characterization of these samples concerning pH, temperature, and total suspended solids (TSS) and volatile suspended solids (VSS) was performed following standard methods [31].

2.3. Isolation and enumeration of E. coli

The following procedure was adopted for the enumeration of E. coli through membrane filtration: 1.00 mL of sample was filtered through 0.45 µm nitrocellulose membrane; then it was placed on a plate containing TBX agar (Oxoid) and incubated at 37 °C for 24 h; finally, the number of positive (blue-green) colonies on the plate were counted. The incubation temperature was 37 °C. The plates prepared with TBX agar were stored at 4 °C for 1–4 days before use.

All blue-green colonies were incubated in a Fluorocult broth at 37 °C for 24 h and the green tubes indol positive were considered as E. coli.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the E. coli isolates was tested for eight antimicrobials using disc diffusion (Kirby–Bauer) method following the Clinical and Laboratory Standards Institute (CLSI, 2012) criteria. This technique consists in putting the disk containing the antibiotic in direct contact with the medium (Mueller-Hinton from Liofilchem) previously inoculated with the E. coli strains isolated as described in section 2.3. The disks used contained the following antimicrobials: erythromycin (15 µg); azithromycin (15 µg); clarithromycin (15 µg); ofloxacin (5 µg); ciprofloxacin (5 µg); sulfamethoxazole (50 µg); trimethoprim (5 µg); and metronidazole (50 µg).

After an overnight incubation period at 37 °C, the inhibition halos were measured with a certified electronic digital caliper (Paget Trading Ltd., Woodrow London SE18 5DH, UK) and compared to standard halos, allowing the results to be categorized. For the E. coli/antibiotic combination there are two breakpoints (marked in Figures 1–7, for each antibiotic), which are the boundaries of the 3 categories of interpretation: susceptible (in vitro inhibition by an antibiotic concentration that is associated with a high likelihood of therapeutic success), intermediate (uncertain probability of successful treatment), resistant (high likelihood of therapeutic failure) [32]. The breakpoint system used was proposed by the European Committee of Antimicrobial Susceptibility Testing, and described by Tascini et al. [32]. The standard deviations of the sample replicates (n=3) represented in Figures 1–7 were considered in the comparison with the breakpoints.

3. RESULTS

3.1. Monitoring of parameters inside the biological reactors

The values of operational parameters monitored inside the biological reactors of the WWTPs, pH, temperature, TSS and VSS (Table 1), indicate normal operational conditions in both WWTPs. Comparing these values with the typical values for operation of an activated sludge biological reactor operating under an extended aeration regime [25]: i) the pH is in the typical range 6–9, near a neutral pH, where optimal performance occurs; ii) temperature values are in the typical range of 10–30 °C for psychrophilic microorganisms, 18.8–23.9 °C and 15.0–21.0 °C respectively for WWTP1 and WWTP2, nevertheless occasionally it is outside the optimal range, 12–18 °C; iii) TSS values should be in the typical range 2000–5000 mg/L but the values are lower than 2000 mg/L, however a healthy percentage for the ratio VSS/TSS (typically equal or greater than 75%) was observed, being higher than 87 and 85% respectively for WWTP1 and WWTP2 (VSS represents the live bacteria portion of solids) [25].

Table 1. Parameters monitored inside the biological reactor for WWTP1 and WWTP2.

	Date	pH	T (°C)	TSS (mg/L)	VSS (mg/L)	VSS/TSS (%)
Biological reactor of WWTP1	23/03/15	7.18	16.8	2030	1810	89.2
	07/04/15	7.29	19.2	2080	1900	91.3
	10/04/15	7.22	18.2	2160	1960	90.7
	20/04/15	7.34	18.9	1950	1700	87.2
	27/04/15	7.05	18.7	1710	1550	90.6
	04/05/15	7.11	18.7	1310	1230	93.9
	11/05/15	7.09	21.0	1560	1470	94.2
	18/05/15	7.07	22.1	1240	1190	96.0
	25/05/15	7.16	22.8	1250	1170	93.6
	01/06/15	7.27	22.1	1420	1350	95.1
	08/06/15	7.33	23.9	1410	1300	92.2
	15/06/15	7.29	21.7	1660	1510	91.0
Biological reactor of WWTP2	23/03/15	6.65	15.5	1040	1030	99.0
	07/04/15	7.06	16.9	970	960	99.0
	10/04/15	6.85	18.5	980	870	88.8
	20/04/15	7.32	15.0	2170	1850	85.3
	27/04/15	6.98	16.4	1970	1680	85.3
	04/05/15	6.12	18.2	1720	1490	86.6
	11/05/15	6.51	19.1	1930	1710	88.6
	18/05/15	6.47	18.7	1550	1380	89.0
	25/05/15	6.52	20.7	1790	1560	87.2
	01/06/15	7.12	21.0	1840	1570	85.3
	08/06/15	7.24	20.2	1550	1360	87.7
	15/06/15	7.38	20.3	1930	1720	89.1

3.2. Analysis of the resistance to the antibiotics of macrolides class

The macrolides, specifically erythromycin, azithromycin and clarithromycin, are the antibiotics most frequently detected in WWTPs [22]. It is noteworthy that erythromycin, clarithromycin and azithromycin are included in the EU first watch list in the Directive 2015/495/EU [33]. These class of antibiotics, bind to the 50S ribosomal subunit with a specific target in the 23S ribosomal RNA molecule and various ribosomal proteins [13]. However, the resistance profile of the E. coli to the three selected antibiotics of this class has some differences (Figures 1–3).

The overall picture of the E. coli in the two WWTPs shows several situations of resistance to erythromycin both in the influent and effluent of the biological reactor (Figure 1). The most critical case for erythromycin was on the 27th of April on WWTP1, for which no zone inhibition was evident from the effluent samples, although the strains from influent showed a halo with 15.0 mm ± 0.3. Moreover, it was also visible a significant decrease (from the influent to the effluent) of the inhibition zone below the resistant limits, on the 7th, the 20th and the 27th of April (Figure 1). The resistance to this macrolide in environmental samples can be justified by the ubiquitous presence of this antibiotic in the aquatic environment, from the Antarctic [34] to the Atlantic Ocean [35], and in wastewaters [31]. From March to June, 50% of the effluent samples analyzed, and released into the environment, have E. coli strains considered erythromycin resistant.

Concerning WWTP2, during April the E. coli strains from the influent and effluent showed halos lower than the value considered resistant. During these three months of campaign, 42% of the total effluent analysis indicate resistance to erythromycin.

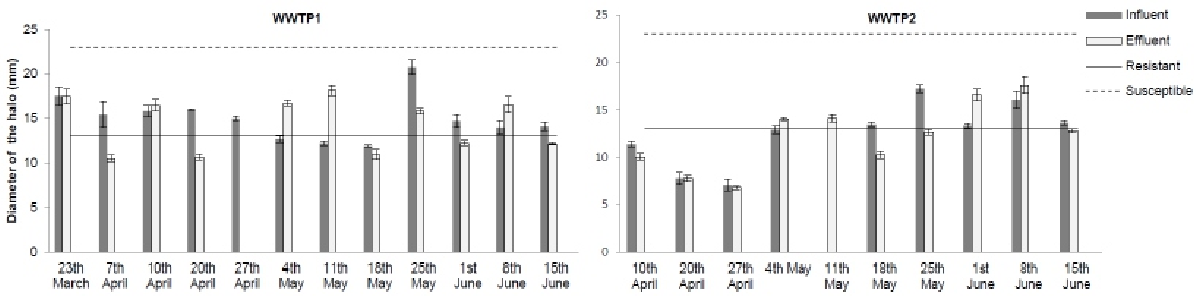

Figure 1. Erythromycin resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

Azithromycin has been described as more effective than erythromycin for many genera of the family Enterobacteriaceae [36]. This is in agreement with the results presented, where 100% of E. coli strains from the effluents of WWTP1 and WWTP2 are susceptible to azithromycin. The inhibition zones that resulted from the exposure to azithromycin discs are among the largest dimensions of this study, the inhibition zones ranged from 32.3 to 18.4 mm (Figure 2). On the 27th of April, in WWTP1 a noticeable decrease of the inhibition zone was identified, from 29.5 to 18.4 mm. The only concerning situation in WWTP2 has occurred on the11th of May, when the strains isolated from the influent of the biological reactor were considered as resistant (Figure 2). The results suggest that there was an entrance of bacteria azithromycin resistant, but they did not persist after the biological treatment of WWTP2.

About clarithromycin evaluation (Figure 3), 25% of influent and effluent analysis in WWTP1 were considered susceptible. In WWPP2 none of the influent samples was considered susceptible and only 8.3% of the effluent samples were considered susceptible (Figure 3).

In the same day (27th of April) antibiotic resistance occurs for both macrolides, clarithromycin and erythromycin, showing the same pattern, which suggests that a common resistance mechanism for these two antibiotics may occur. Also, on the 20th of April, in both WWTPs, and on the 27th in WWTP1, the strains from the effluent reveal no susceptibility to clarithromycin. This may suggest an acquired resistance during the biological treatment. The opposite occurs in WWTP2 on the 11th of May. The E. coli strains exhibit a higher resistance to erythromycin than to the other two tested macrolides, showing 33.3% of resistance in the analysis of WWTP1 and 37.5% in WWPT2. Several reports identified resistance to clarithromycin associated to mutations on the positions A2058 or A2059 of the 23S rRNA [37].

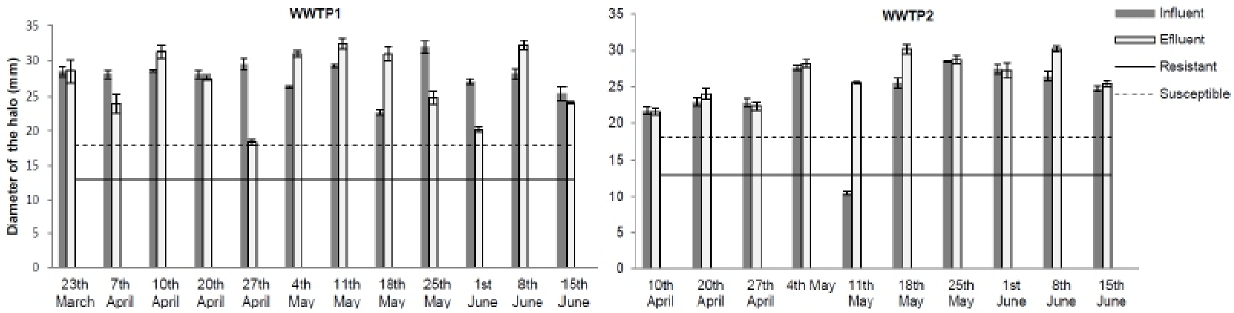

Figure 2. Azithromycin resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

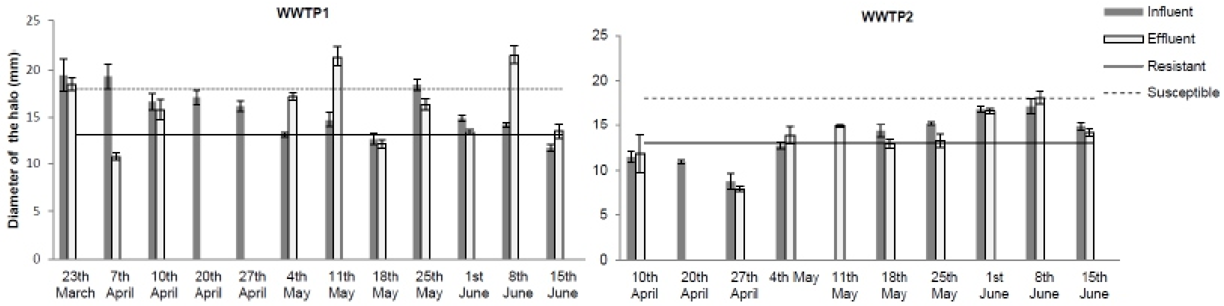

Figure 3. Clarithromycin resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

3.3. Analysis of the resistance to the antibiotics of quinolones class

The quinolones act by converting their targets, gyrase and topoisomerase IV, into toxic enzymes that fragment the bacterial chromosome. The susceptibility of the E. coli isolated from the two WWTPs to quinolone antibiotics was also tested. Ofloxacin and ciprofloxacin, which are from the second generation of quinolones (with the introduction of a fluorine), displayed a considerably improved activity against gyrase, and enhanced pharmacokinetics and pharmacodynamics [38]. An oscillation of sensibility to ofloxacin (Figure 4) is observed before and after the biological treatment of WWTP2 in March and April, and in WWTP1, from April to May. In the end of May and June both WWTPs showed halos >16 mm in the influent and effluent of the biological reactor, thus these strains were classified as susceptible. The most critical situations of resistance were identified on the 27th of April in WWTP1 (without halo at the entrance) and on the 18th of May in WWTP2, where the resistance deeply increases during the biological treatment (Figure 4).

Ciprofloxacin and ofloxacin are effective for treating infections caused by many different types of bacteria even for antibacterial prophylaxis [39]. Ciprofloxacin was the first quinolone that displayed significant activity outside of the urinary tract. After almost three decades in clinical use, ciprofloxacin remains one of the most commonly prescribed antimicrobial drugs, being listed by the World Health Organization (WHO) as an essential medicine and a critically important antibiotic [40]. So, these antibiotics are in the list of the main antibiotic compounds detected in the influent and effluent of WWTPs [22]. The removal of some antibiotics was investigated in six WWTPs in Italy during summer and winter. It was found that similar removal efficiencies were obtained for ciprofloxacin and ofloxacin in both seasons, 60% and 50%, respectively [41]. Varela et al. reported that the percentage of Enterococci isolates resistant to ciprofloxacin was 4.0% in the influent, while it decreased to 3.4% in the effluent [42].

In this study (Figure 5), a significant resistance increase is observed after the biological treatment in several situations: on the 23th March, and on the 1st June in WWTP1; and on the 10th April, on the 18th and on the 25th May in WWTP2.

Looking at the results obtained for ciprofloxacin and ofloxacin (Figures 4 and 5), E. coli resistant (to both) antibiotics was discharged into the environment only in two days in WWTP1 (23th March and 27th April) and in one day in WWTP2 (18th May). However, the spread of these resistant microorganisms may pose environmental and health risks. The acquisition mechanisms of quinolone resistance has been described as: i) chromosomal mutations in genes encoding the protein targets, or mutations causing reduced drug accumulation, either by a decreased uptake or by an increased efflux; or ii) plasmid - located genes associated with quinolone resistance [43].

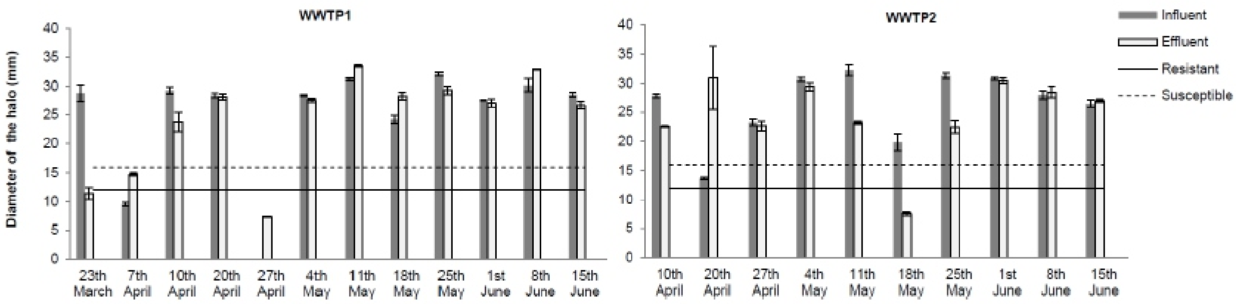

Figure 4. Ofloxacin resistance of E. coli in the influent and effluent of the biological reactors in WWTP 1 and WWTP2.

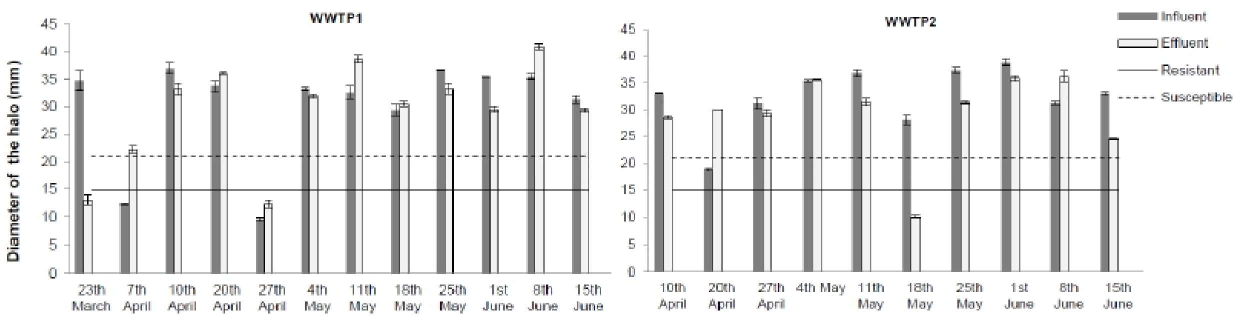

Figure 5. Ciprofloxacin resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

3.4. Analysis of the resistance to the antibiotics sulfamethoxazole and trimethoprim

Trimethoprim and sulfamethoxazole are synergistic folate pathway inhibitors. The combination of trimethoprim and sulfamethoxazole is widely used not only for therapy but also for prevention of infections. The combination trimethoprim/sulfamethoxazole is recommended for use both in adults and children for several infections and as prophylactic. Trimethoprim affects the bacterial folic acid synthesis by the inhibition of dihydrofolate reductase (DHFR). Bacteria may become resistant to trimethoprim by several mechanisms: the development of permeability barriers, efflux pumps; the existence of naturally insensitive target DHFR enzymes; mutational and regulation changes in target enzymes; and the acquirement of drug-resistant target enzymes [44].

Although in the WWTP1 in 50% of the strains (isolated on the same day) from influent and from effluent were susceptible to trimethoprim (Figure 6), several critical situations were identified: on the 7th of April no halo was observed in both influent and effluent samples; a deep increase of resistance was noticed from the 10th to the 27th of April, when the E. coli strains from the influent were susceptible, while the strains from the effluent showed resistance (without halo). This suggests a resistance dissemination during the WWTP1 biological treatment. After the 4th of May, in the seven following campaigns until the 15th of June, the E. coli strains from effluent were susceptible to trimethoprim.

Regarding the WWTP2, the same pattern is observed, 50% of the strains (isolated on the same day) from influent and effluent were susceptible to trimethoprim (Figure 6). However, several critical situations (without halo) were observed, on the 20th of April and the 11th of May in the influent and, the most concerning situation occurred on the 10th of April when the strains isolated from the effluent do not exhibit any halo.

Sulfamethoxazole inhibits the production of dihydrofolate from para-aminobenzoic acid. Usually, the antimicrobial susceptibility tests are performed with trimethoprim or trimethoprim /sulfamethoxazole. According to the authors best knowledge, information is not available regarding the susceptibility halo of SMX for 50 µg disks.

In both WWTPs (Figure 7), the samples of E. coli strains isolated from the influent and effluent, in the same day, that showed susceptibility to sulfamethoxazole represent 50% of the samples analyzed. In WWTP1, the E. coli strains isolated from the influent and effluent demonstrated resistance (without halo) to sulfamethoxazole on the 7th, on the 27th of April, and the 8th of June (Figure 7). Between the 10th and the 27th of April, despite the influent strains showed susceptibility, resistance (without halo) was exhibited in the strains isolated from the effluent in these same days, which represents a critical increase of resistance. The same has occurred in the WWTP2 on the 10th of April (Figure 7), being considered the most critical situation.

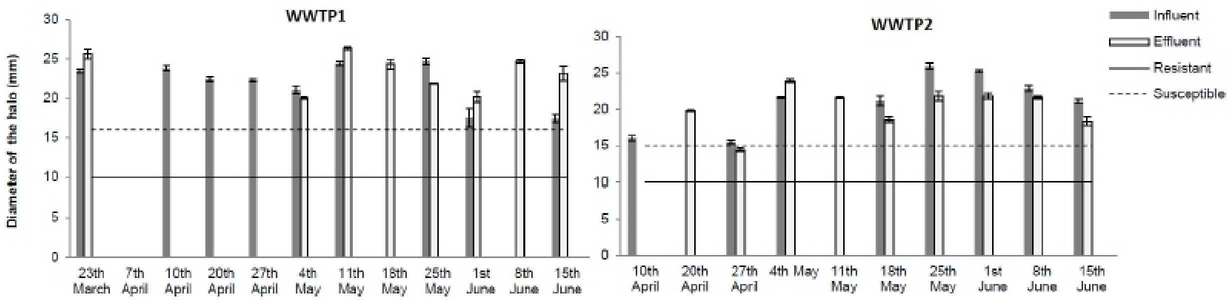

Figure 6. Trimethoprim resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

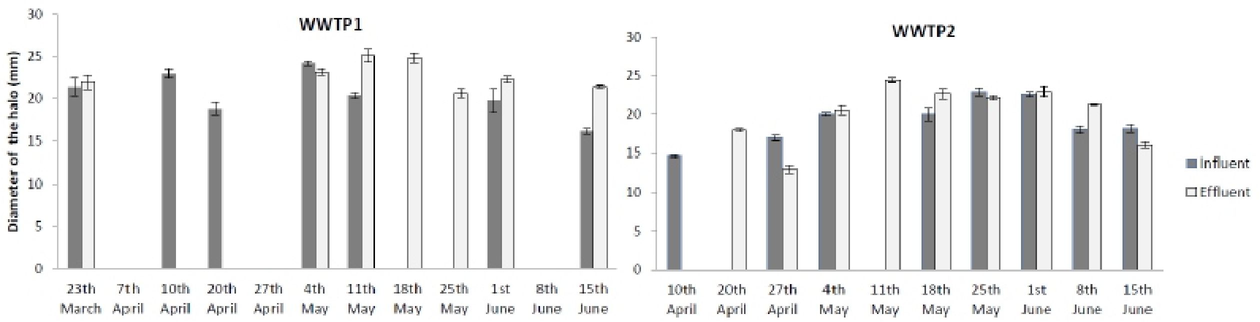

Figure 7. Sulfamethoxazole resistance of E. coli in the influent and effluent of the biological reactors in WWTP1 and WWTP2.

3.5. Analysis of the resistance to antibiotic metronidazole in the WWTPs

Metronidazole is described to have some activity against coliforms in vivo, despite showing in vitro resistance [45]. According to the fact that both WWTPs own aerobic processes is not predictable the occurrence of resistant strains to metronidazole. However, E. coli strains with lesions in their DNA repair systems may show a decrease in minimal inhibitory concentration [44]. Therefore, it is pertinent to evaluate the sensibility of E. coli to this antibiotic. But, as expected, none of these strains exhibited resistance in the tests performed.

3.6. Overview of antibiotic resistance

Different behaviors were observed during this study in both WWTPs, namely variability of resistance from antibiotic to antibiotic, along with time, from WWTP1 and WWTP2, and increase or decrease of resistance when comparing the E. coli isolated from influent and effluent. The different behaviors reported may be related with different factors that interact, which are described below.

Wastewater contains substances that may exert an array of effects on bacteria, being sometimes designated as stressors, of which are examples the antibiotic residues and metabolites thereof. These stressors may also shape the surviving community because different organisms, or groups of related organisms, have different degrees of tolerance, or defensive responses, against their adverse effects [18,46]. So, depending on the type of antibiotic that may be present in the WWTP it may cause different resistance behavior in influent and effluent.

The difference of resistance observed may also be related with the microbiota (mainly composed of human commensal bacteria) present at that time, which is mixed with bacteria from different origins that may be entering and colonizing the sewage treatment system [18]. In this environment, the fraction of ARB may reach more than 50% at least within a given group (e.g., enterobacteria or enterococci) [18,46].

In the secondary treatment the fraction of ARB&ARG gets in contact with the microorganisms present in the biomass suspension, which submitted to the potential selective pressure of antibiotics present in the inflow increase the potential of ARB&ARG's dissemination.

The sludge recirculation, from the secondary sedimentation tank to the biological activated sludge reactor, ensures enough cells to reduce the organic load of the wastewater. The microbiota (including ARB) arriving to the biological reactor is stimulated to compete with the activated sludge bacteria for the available organic matter. This intense metabolic activity creates an important bacterial community dynamics. The shifts occurring in the bacterial community, the fitness of ARB, and the success with which their ARGs are spread to other bacteria are crucial to the ARB&ARG's dissemination [18].

Since municipal WWTPs are the direct link to the aquatic environment, and they are not designed to eliminate ARGs and ARBs, they promote the spread of ARGs in allochthonous bacteria [47]. This study supports the need of advanced combined treatments to prevent the dissemination of ARG and ARB as recommend by the World Health Organization.

3.7. Efficiency of disinfection

The revised standards of World Health Organization for unrestricted irrigation of treated wastewater were 1000 fecal coliforms /100 mL (monthly mean) although a more stringent guideline (200 fecal coliforms / 100 mL) was considered more appropriate for public lawns with which the public may come into direct contact [48]. More recently the United States Environmental Protection Agency has included effluent limits for E. coli, rather than fecal coliforms, in order to protect the use of primary contact recreation in the receiving water: the monthly geometric mean concentration of E. coli must not exceed 126 organisms per 100 mL, further, no single sample may exceed 406 organisms per 100 mL (instantaneous maximum limit) [48]. However regulations for disinfection are site-specific, have seasonal standards, and are under continual review [25].

Disinfection is mandatory in WWTP2 because it discharges into a sensitive zone, but in this case only in the hot season and there is no legal limit imposed. Disinfection efficiency was evaluated through E. coli count before and after ultraviolet (UV) irradiation.

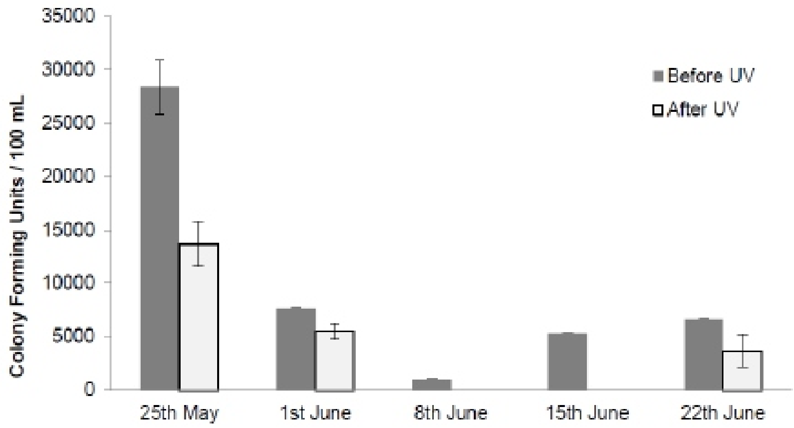

Figure 8. UFC of E. coli per 100 mL before and after disinfection by UV radiation in WWTP2.

The efficiency of the disinfection treatment using UV radiation from the 25th of May to the 22nd of June is illustrated in Figure 8. The removal of bacteria from treated wastewater was complete (the counted values were lower than the limit of detection) on the 8th and the 15th of June, but it should be noted that the initial concentration was quite low. For the other tested days, the efficiency was close to 50% and the concentration after disinfection was much higher than 406 bacteria per 100 mL [48]. In fact the inability to achieve permit limitations is the most common problem related with UV disinfection, which may be due to: hydraulic problems related with the creation of density and/or eddy currents and/or dead volumes that cause short circuiting and therefore reduce the contact time leading to ineffective use of the UV system, which is difficult to detect and needs the help of studies of computational fluid dynamics; formation of biofilms on the exposed surfaces of the UV reactor, which demands frequent cleaning using a cleaning agent or disinfectant to remove them; presence of particles that disperse the radiation, that should be prior removed by filtration [25]. Although the disinfection system by UV radiation is efficient it has been demonstrated that it promotes the spread of ARGs mediated by conjugative transfer of plasmids between E. coli strains [49]. Therefore, special care should be taken in the use UV radiation for disinfection.

4. CONCLUSIONS

The results from this work contribute to the aware of ARB dissemination from WWTPs. The evaluation of the antibiotic susceptibility of E. coli strains isolated from the influent and effluent of the biological reactors in two WWTPs allowed the identification of several critical situations (e.g., erythromycin, clarithromycin, and trimethoprim): i) occurrence of bacteria resistant after the biological treatment, having as consequence the discharge in the aquatic environment of resistant strains; ii) the identification of extreme resistance (zero halo of antibiotic's diffusion), in the influent and/or effluent of the biological reactor, being the last the most critical situation; iii) and the prevalence of the resistance over time.

The analysis performed before and after the UV irradiation system revealed that the disinfection processes contributed to the decrease of bacteria number released in this WWTP. However, a reduction of the bacteria number released does not mean a reduction of ARB because UV irradiation may promote the conjugative transfer of plasmids between E. coli strains. Reuse of treated wastewater demands stringent requirements in order to avoid potential environmental and public health impacts. The use of other disinfection system, such as ozonation, would be a possible alternative to avoid the spread of ARB both in the aquatic environment and soil, when treated wastewater is reused, namely for irrigation, reducing the potential risks for environmental and public health.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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Spatial and Statistical Characteristics of Heat Waves Impacting India

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ABSTRACT

The climate of a place has a decisive role in human adaptations. Man's health, adaptability, behavioural patterns, food, shelter, and clothing are mainly influenced by the temperatures of the area. Hence, a study is undertaken to analyse the spatial distribution, frequency, and trend in the heat waves over the country. The statistical characteristics of heat waves over India are addressed in this study. Gridded daily temperature data sets for the period 1951–2019 were used to compute the arithmetic mean (AM), standard deviation (SD), coefficient of variation (CV), and trends of monthly maximum temperature. The number of heat wave days were identified using the criteria given by India Meteorological Department (IMD) i.e., a heat wave is recognized when the daily normal maximum temperature of a station is less than or equal to (greater than) 40 °C than it will be considered as a heat wave if the daily maximum temperature exceeds the daily normal maximum temperature by 5 °C (4 °C). The analysis was confined to the two summer months of April and May only. The spatial distribution of the AM shows higher values during May, and the core hot region with temperatures exceeding 40 °C lies over central India extending towards the northwest. The SD distribution shows higher values over the northeast of central India decreasing towards the southwest. The CV distribution shows higher values over the north decreasing toward the south. Higher numbers of heat waves are observed during May and the number is higher over Andhra Pradesh and south Telangana regions of southeast India. This study concludes that a moderate hot region experiences a higher number of heat wave days over India.

Keywords: heat waves; maximum temperatures; climate; statistical metrics; trends

1. INTRODUCTION

The climate of a place has a say in human adaptability. The people in an area make suitable adaptations of food, shelter, and clothing, etc., in commensuration with the perceived climatic conditions of that place. Despite all these modifications, the extremes in temperatures and humidity can lead to discomfort, health issues, and morbidity. A prolonged period of higher maximum temperatures than the adaptable values of maximum temperatures in hot weather season can lead to what is known as heat waves. Heat waves can aggravate dry and drought conditions. Increasing urbanization and industrialization and unbridled development leads to increasing temperatures trapped in concrete structures what we call the urban heat island effect. As the global mean surface air temperatures have escalated extreme weather events and natural hazards such as heat waves, excess precipitation events, droughts etc. have amplified in intensity, durability, and frequency [1,2].

The extreme weather event heat wave is every so often referred to as the "silent atmospheric killer" and this is strongly connected to anthropogenic climate warming especially in areas where senior citizens reside [3–5]. The disaster due to this heat wave is high, for example during 2003 in Europe higher than 70000 death rates were reported due to heat wave and in Russia 2010 above 50000 deaths occurred [1]. A global analysis conducted since 1980 on deadly heat waves and observed that nearly 30% of the population is presently at threat as the heat wave thresholds are increased. Not only daytime maximum temperatures hazardous to human health and life but also warm minimum temperatures during night time also have a great concern on the mortality rate and causing unfavourable conditions to human health during the last few decades across the globe and also durability of heat wave also plays a crucial role for example, one day of extremely hot conditions will not produce severe impacts but if the situation persists for more than two or three days causes a major threat [6]. There are increases in both land and ocean temperatures besides more frequent heat waves in most land regions. The Intergovernmental Panel on Climate Change (IPCC) report also underlined an increase in the frequency and duration of marine heat waves as a consequence of global warming and also the surface mean air temperature has amplified by 0.7 °C from 1961 to 2000 [7].

A heat wave is a protracted period of extremely high temperatures or hot weather as generally perceived and scientifically defined as the occurrence of temperatures higher than normal temperatures over the region. Heat waves are known to be responsible for the highest number of human deaths of all-weather related disasters [8,9]. A decreased trend in temperature over Northern India and an increasing trend in temperature over Southern India was observed [10]. Earlier when there is a lack of availability of data trends of both temperatures i.e., maximum and minimum during 1880 to 1950 were studied and consummate that there are no regular changes in both the temperatures [11].

In the20th century the annual mean temperature datasets of 73 stations over India showedan increase of 0.4 °C [12]. Heat waves affect human health in many ways, cause hyperthermia when the temperatures are higher than 40 °C which leads to heat oedema; heat rash; heat syncope and heat exhaustion, and sometimes death. Reports indicate psychological stress in addition to physical stress due to extreme temperatures [13]. Abnormally high temperatures also have societal impacts such as exacerbating conditions leading to power outages, wildfires, melting of roads, buckling and kinking of rails, bursting of water lines etc. [14]. Three vulnerable zones and heat waves zones were identified over Indian subcontinent [15]. In recent decades, the concern about extreme high temperatures and heat waves had increased due to the observed increased tendency in their frequency and severity since 1950 and an indication of their continued increase in future due to global warming climate change [7]. These are evident from the recent occurrences of long and severe heat waves over western and central Europe in August 2003 and July 2006 that are associated with high human mortality [8,16,17]. The indications of enhanced frequency, intensity, and duration of heat wave conditions from future climate projections raise concerns not only on the human mortalities but also human activities with socio-economic impacts [7,18]. From a statistical point of view, a small change in the mean value of a climate variable (such as temperature) corresponds to large changes in weather, which means a small shift in the mean of temperature distribution imply a sizeable change in the frequency and intensity of temperature extremes [19]. The variations of weather parameters (such as temperature) may be described as a bell-shaped curve, meaning that normal weather is common and weather extremes are rare. In this situation, a small increase in temperature shifts the entire curve toward hotter high temperatures, meaning that extreme heat events become more severe and more frequent.

Heat waves over India are important due to human mortality associated with them and the increase in the mortality rate in recent years [20]. Human deaths in India due to heat waves are noted to be 1425, 1393, and 2500 during 2003, 2013, and 2015 respectively. Indian subcontinent experiences four seasons, namely winter (December to early March), pre-monsoon (summer, March to June), rainy (or monsoon, June to September), and fall (post-monsoon, October-November). Although the highest maximum temperatures are recorded in May, temperatures exceeding 38 °C are not uncommon in April associated with heat wave conditions. Summers in India are known for the sweltering heat with temperatures exceeding 40 °C. Most of the heat waves over India occur in May as the sun rays reach the surface of the earth directly due to the transition of the Sun in the direction of north resulting in the accretion of heat in the north-western parts of the country and the physical patterns of the central plateau and northern plains [21]. Northern India is familiar with heat waves annually but the south-eastern parts of the country experiencing since the decade 1970s [22]. Distribution of temperature during the pre-monsoon season represents that the maximum temperature is located over the north-western parts of the country as the wind blows from Middle East Asia bringing hot and dry air to north-west India through Pakistan and from there the hot air passes to central and south eastern parts of the country representing the advection of temperature [23,24].

The characteristics of heat waves differ from region to region, in terms of the climate conditions describing the maximum temperatures of the region, e.g., the heat wave in Europe during 2003 continued for nearly three months i.e., from June to August [25,26], while in the United States during 1995 and 1999 in July persists only for a few days [27,28]. Different studies were made on heat waves over different parts of the globe and given different threshold to define heat waves for example over Australia [29], USA [30], and Europe [31]. Over India the synoptic features associated with heat wave were studied, and proposed that the reason behind high temperatures and the source of heat is from the Thar Desert and Rajasthan [32–34]. A few studies attempted to describe the characteristics of heat waves over India. Heat wave days over India had been increasing during 1961–2010 and that the increasing trend is more during the warmer decade of 2001–2010 and observed that the highest anomaly of 6–8 °C of composite temperatures was seen over coastal Andhra Pradesh reflecting this to be the vulnerable region to heat wave [35]. Heat wave over Karachi, Pakistan during June 2015 recorded a maximum temperature of about 44.8 °C and heat index of about 66 °C and reported a death toll of about 1200 [36]. A report from the National Disaster Management Authority (NMDA), Government of India [20] stated that the frequency of heat wave days is 5 to 6 over India. The NDMA proclaimed that there is an increasing trend in number of heat waves. The report also added that the deaths due to heat wave during period from 1992–2015 were about 22562.

In India, the occurrence of a heat wave is recognized according to the criteria given by India Meteorological Department (IMD). A heat wave is declared (i) if the mean maximum temperature of a station is ≥ 40 °C for plains and hilly areas ≥ 30 °C and the departure from the normal maximum temperature is 4–6 °C (ii) To be declared as a severe heat wave if the mean maximum temperature of a station is ≥ 40 °C and the departure from the normal maximum temperature is greater than 6 °C or if the station's normal maximum temperature is ≥ 47 °C [37].

The present study is an attempt to describe the statistical characteristics of heat waves over India using gridded temperature data for the period from 1951 to 2019. This study brings out the spatial distributions of the statistical properties such as the arithmetic mean, variability, and trends of temperatures and heat wave days.

2. Data and methodology

2.1. Data

To study heat waves one of the most efficient and high resolution data set established by India Meteorological Department is the daily temperature gridded data set. By using 395 synoptic weather stations which were spread evenly over the Indian subcontinent this data set was developed using daily maximum temperature i.e., during afternoon hours. Shepard's angular distance weighting algorithm was used to interpolate station data into regular grids. Daily maximum temperature anomalies were used instead of absolute values to avoid biases. The climatological normal of maximum temperatures were calculated for every station for the period 1971–2000. This technique of interpolation needs a considerate understanding of the spatial correlation structure of the station data. For this reason, correlations of inter-station were evaluated to examine the distances over which maximum temperature anomalies are connected. The station data was first subjected to primary quality controls such as confirming uniformity and eradicating outliners. Moreover, it was also confirmed that the data length of all the stations should have a similar length to evade errors caused due to inhomogeneity in station density in a gridded dataset. In the beginning, this gridded dataset was established for the period from 1969 to 2005 and later restructured for the period from 1951 to 2019 and for the present study 69 years of a gridded dataset (1951–2019) is considered [38]. Meteorological subdivisions over India as per IMD are given in Figure 1.

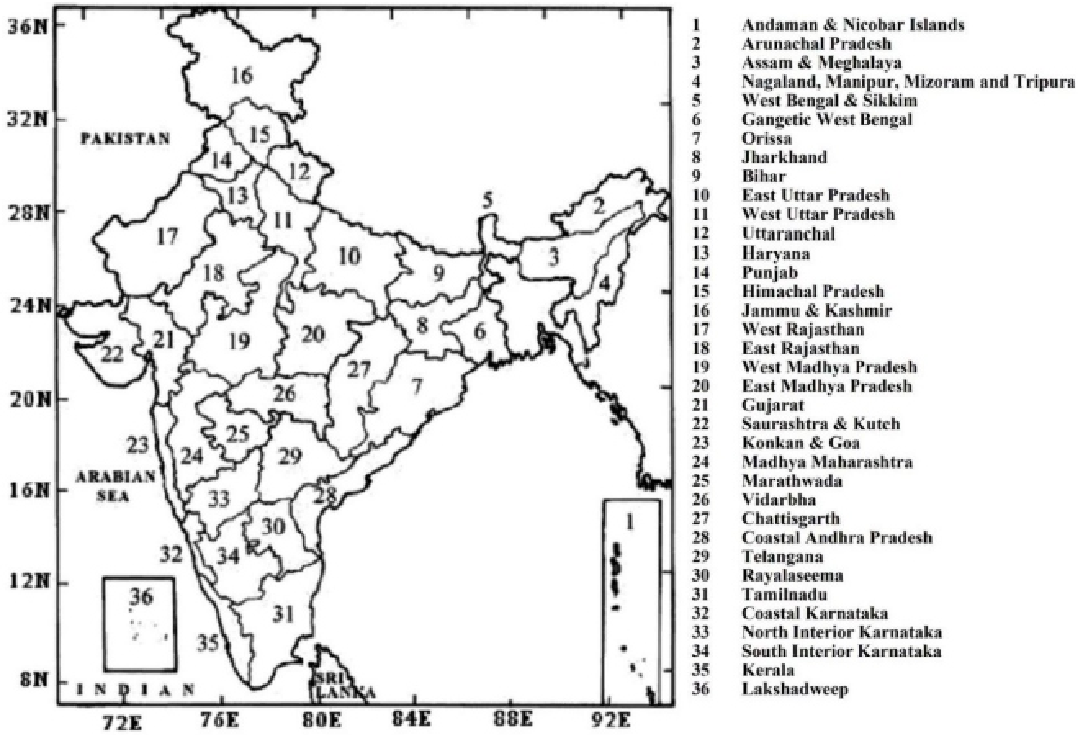


Figure 1. Meteorological subdivisions over India (as per IMD classification).

2.2. Methodology

(I). The Arithmetic Mean (AM), Standard Deviation (SD), and Coefficient of Variation (CV), are calculated for each of the grid points in April and May separately using daily gridded data sets during 1951–2019. The Eqs are

$$Mean\,(\overline{X}) = \frac{\sum X}{N} \tag{1}$$

$$\text{Standard Deviation (SD)} = \sqrt{\frac{\sum\left(X-\overline{X}\right)^2}{N}} \; (\qquad (2)$$

$$\text{Coefficient of Variation (CV)} = \left(\frac{SD}{\overline{X}}\right) \qquad (3)$$

(ii). The number of heat wave days is calculated considering the criterion of greater than mean+4 °C. The number of days is calculated for each of the grid points and calculated separately for April and May.

(iii). To calculate trend a non-parametric rank based statistical test known as Mann-Kendall test was applied to the long term maximum temperature dataset for the period 1951–2019 to determine the significant trends. It is given as follows:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} sig(X_j - X_i) \qquad (4)$$

$$V(S) = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p=1}^{q} t_p\left(t_p-1\right)\left(2t_p+5\right)\right] \qquad (5)$$

In the above Eq Xi and Xj are the time series of observations in chronological order; n is the time series length; tp is the number of values tied for pth value and q represents the number of values tied.

3. RESULTS AND DISCUSSIONS

In this section, some statistical parameters are computed and examined to understand thecharacteristics of heat waves over the Indian subcontinent using the currently available gridded daily maximum temperature datasets (Section 2.1).

The first part of the results describes the basic statistical properties (i.e.,) simple arithmetic mean (AM), standard deviation (SD), and coefficient of variation (CV). In the second part, results considering the number of heat wave days are presented. In part 3, trends in the maximum temperature during the 69 years (1951–2019) are presented.

3.1. Distributions of AM, SD, and CV

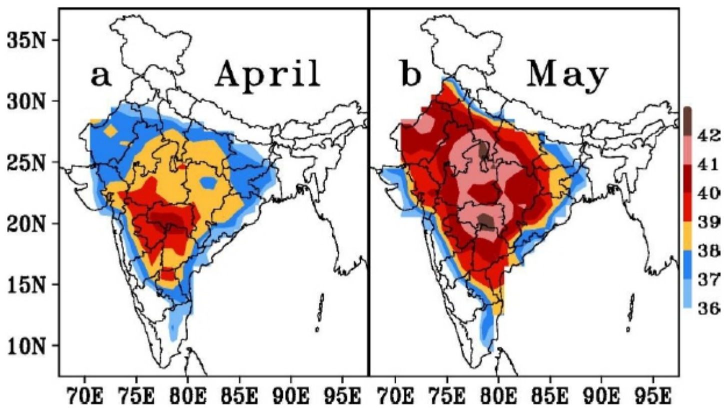


Figure 2. Spatial distribution of monthly mean maximum temperature (°C) over Indian Subcontinent for (a) April and (b) May for the period 1951 to 2019.

The spatial distributions of mean monthly maximum temperature during April and May are presented in Figure 2. During April, the hot region with temperatures exceeding 37 °C exists over coastal parts of India with a small core maximum of 40–41 °C over Vidarbha (Figure 2a). Temperatures are below 30 °C over the west coast, northeast, and north India. During May, the temperature distribution is similar to that of April, except that the temperatures over central India are higher by 2 °C, and the region of the maximum extent from central India to northwest Rajasthan (Figure 2b). The core of maximum temperature still maintains the same temperature in May whereas there is an increase in maximum temperature up to 2 °C from central India to west Rajasthan, where the temperatures are 40–43 °C. However, the regions of the west coast, northeast, and north India are cooler with temperatures below 32 °C.

The distribution of SD shows higher values over the north, decreasing towards the south (Figure 3a). During April the northern region has SD of 1.4 °C to 2 °C; the northern parts of central India have 1.2 °C to 1.4 °C; southern parts and central India have 1 °C to 1.2 °C; the west coast and south peninsula having the lowest SD of 0.8 °C to 1 °C. During April SD is higher over NE India in the order of 1 °C to 1.4 °C whereas it is around 0.8 °C to 1 °C during the May month. Southeastern parts of India i.e., Andhra Pradesh and Telangana are occupied with higher SD values during May of the order of 1.2 °C to 1.4 °C whereas it is 0.8 °C to 1 °C during April. During May the distribution shows a slightly minimum over north decreasing towards the west with comparison to April (Figure 3b). The SD over Gujarat has decreased to 0.8 °C to 1 °C during May while it is 1 °C to 1.2 °C during April and remained alike over the eastern parts of Jharkhand and Bihar in central India. The coastal areas are cooler and wetter than the inland regions and the center of the continents have a large range of temperatures resulting in temperatures being very high and dry as the moisture from the sea evaporates before it extends to the center of the continent resulting in low mean maximum temperature and standard deviation over the coastal regions.

The distribution of CV shows maximum over north decreasing towards the south (Figure 4a,b). The spatial distribution is mostly similar during April and May except that CV slightly increased over a south peninsula and decreased over northeast parts during May. The CV over northern parts of north India is smaller during May as compared to April.

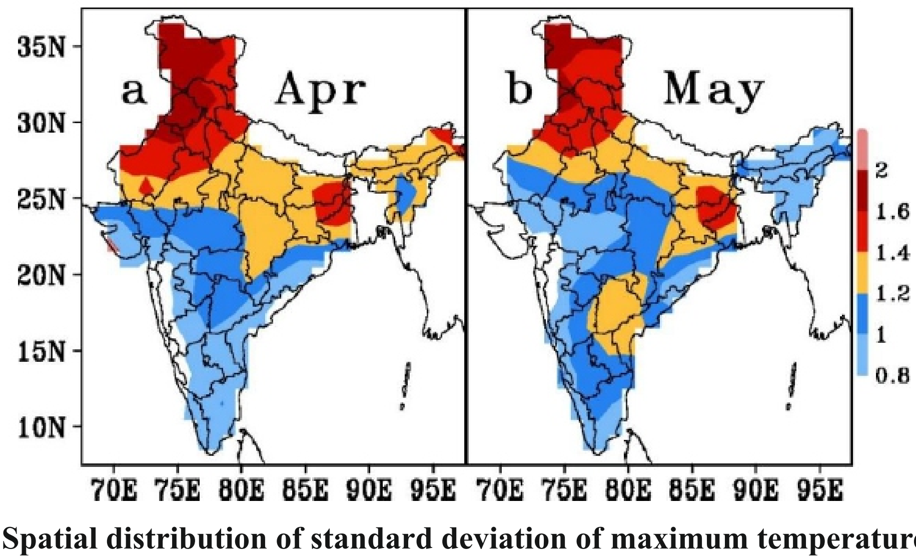


Figure 3. Spatial distribution of standard deviation of maximum temperature (°C) for (a) April and (b) May for the period 1951 to 2019.

The above description of AM, SD, and CV indicate a general pattern of a higher degree of variability over northeastern parts which is cooler; and a slight decrease of CV over the hot region of north central India indicates that hot regions have a lesser CV as compared to higher CV over cooler the region. Most importantly the CV is larger over a south peninsula and southeast coast, specifically over coastal AP which has mean temperatures of 38–40 °C during May had SD between 1.2 to 1.4 °C thus contributing to higher CV over a small region. This indicates frequent occurrences of hot spells over this isolated coastal region.

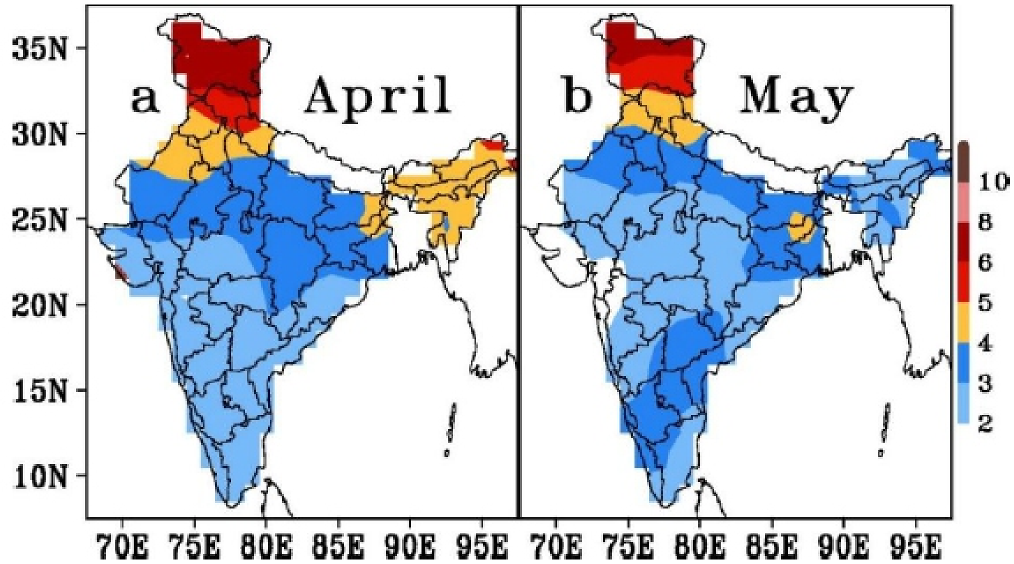
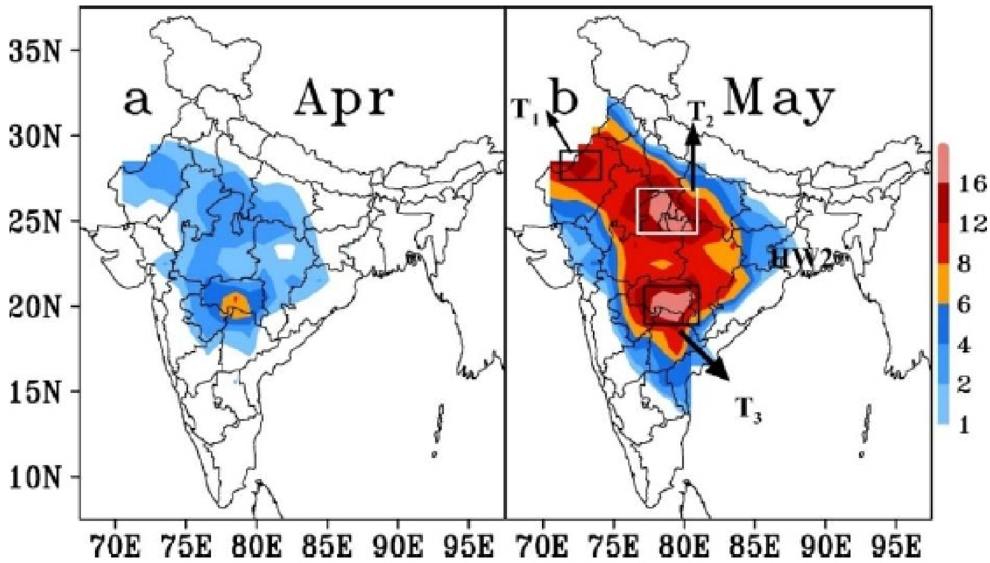


Figure 4. Spatial distribution Coefficient of Variation of monthly maximum temperature (°C) for (a) April and (b) May for the period 1951 to 2019.

3.2. Distribution of maximum temperature days above 42 °C

Spatial distribution of days with maximum temperatures exceeding 42 °C were plotted from 1951–2019 for the hottest months of April and May. In April, a small isolated region over Vidarbha has the highest frequency with more than 6 days/year and it is the hottest region when compared to the rest of the country (Figure 5a). The Frequency of 4–6 days occurs over South Telangana, Marathwada, West Madhya Pradesh, and West Uttar Pradesh. The frequency of 1–2 days is noted over West Orissa, Chhattisgarh, East & West Madhya Pradesh, East & West Uttar Pradesh, East & West Rajasthan, Madhya Maharashtra, South Telangana, Rayalaseema, and Gujarat. During May, the area extent and frequency of days were larger and three hottest temperature zones were observed i.e., West Rajasthan (T1), Northwest Madhya Pradesh (MP) (T2) and Southwest Uttar Pradesh (UP) (T3), and East Maharashtra [15] (Figure 5b). In May, the frequencies are the highest (above 16) over Vidarbha and North Telangana; 12–16 over East Madhya Pradesh, Rajasthan; 1–22 over Chhattisgarh; 16–22 over West Uttar Pradesh; 18–26 days over South Telangana and East Uttar Pradesh; 14–22 over Bihar; 8–16 over Gangetic West Bengal; 6–16 over Coastal Andhra Pradesh; 4–14 over Orissa; and are below 4 over rest of the country (Figure 5b). It is important to note that Vidarbha and Telangana have the highest frequency of above >26 days in May whereas Rajasthan has the frequency of 20–26 days. From the spatial distribution plot, it is clear that higher frequency regions coincide with maximum temperature regions.

The identified maximum temperature region of East Maharashtra is noted as the hottest. Days with a highest average frequency of 14–20 days were noted over the zone of "hot region" during the May month and from the spatial distribution plot, it is clear that the regions of the south-central parts are the warmest when compared to the rest of the Indian subcontinent. This finding of the isolated hot region [39] will be useful to comprehend the occurrence of heat waves over southeast coastal regions. Temperatures over Vidarbha and Telangana are higher than elsewhere, not only because of the remoteness from the sea but also due to the black soil of the area [39–40].



5. Spatial distribution of the number of days above 42 °C days in (a) April and (b) May for the period 1951 to 2019.

3.3. Distribution of heat wave days

Heat wave days as mentioned earlier, are calculated using the criteria that a heat wave day is identified when the maximum temperature exceeds the mean value by 4 °C. For brevity, it is stated that the maximum temperature of each day of April and May is compared with the respective daily mean maximum temperature and the occurrence of heat wave day is identified. The spatial distribution of the count of heat wave days during April and May is presented in Figure 6 for the period 1951 to 2019. During April, the highest number of heat wave days is observed over north India decreasing towards the south (Figure 6a). The region with heat wave days greater than 20 days is confined to only northern parts at locations higher than 25oN and over east-central India at latitudes higher than 22o N and east of 82o E. The heat wave days were maximum over west Rajasthan, Jharkhand, and Bihar, and no heat wave days were observed over hilly regions, northeast India, west central India. Over peninsular India only, Andhra Pradesh and Telangana are noted to have 5 to 10 heat wave days. Heat wave days are greater in May in a small region of extent (Figure 6b). A remarkable feature that is to be noted is that heat wave days have increased to a higher extent over a small region of Andhra Pradesh and Telangana. The contrastingly located maximum over Andhra Pradesh and Telangana assumes importance as the number of heat wave days rises during May and that took over a region with a maximum temperature at 38–40 °C. Although not presented here, observations indicate that the highest percentage (80–90%) of heat wave related human deaths over India are in Andhra Pradesh and South Telangana.

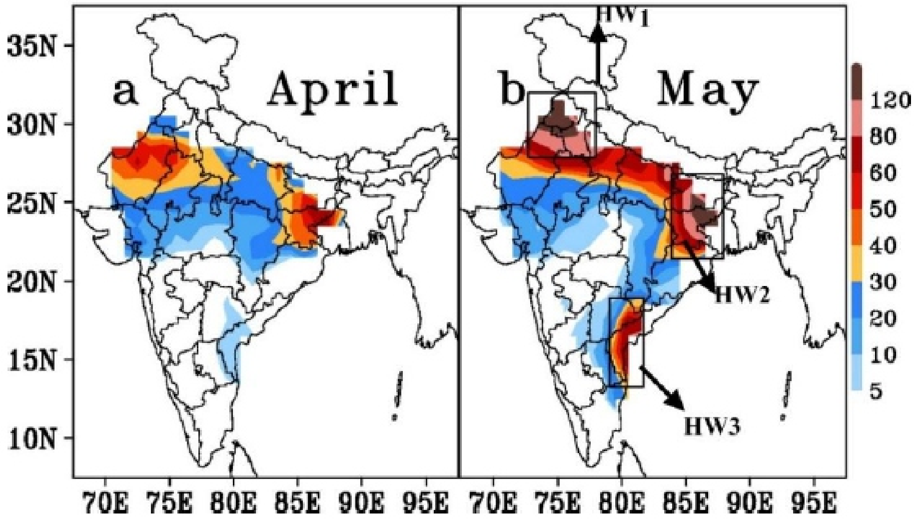


Figure 6. Spatial distribution of heat wave days in (a) April and (b) May for the period 1951 to 2019.

3.4. Spatial distribution of mean minimum temperature

During April, the minimum temperatures above 24 °C were observed in the east coastal region, Telangana, and south Vidarbha (Figure 7a). Around 22–24 °C were observed in some parts of Gujarat, Saurashtra, Kutch & Diu, Konkan & Goa, Madhya Pradesh and southern parts of Chhattisgarh. The mean minimum temperatures were also showing an increasing tendency in May. It is clear that there are two distinct regions of temperatures exceeding 26 °C, which are in east central parts of India (Telangana, Andhra Pradesh, Vidarbha, the southern part of Chhattisgarh and Orissa) and north central parts of India (east Rajasthan, west and east Madhya Pradesh, east UP) (Figure 7b).

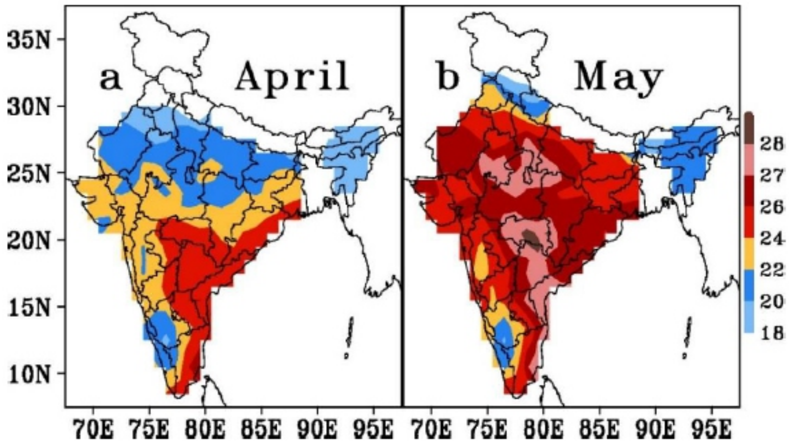


Figure 7. Spatial distribution of monthly mean minimum temperature (°C) over Indian. Subcontinent for (a) April and (b) May for the period 1951 to 2019.

3.5. Climate trends

Trends of maximum temperature, frequency of days with temperatures above 42 °C, and heat wave days were investigated for a period of 69 years (1951–2019). Secular disparities of maximum temperatures over locations of the small region were also examined.

3.5.1. Maximum temperature trend

As a part of the analysis, trends in the monthly mean maximum temperature during the period the 1951–2019 are computed for each of the grid points and for April & May separately. The spatial distribution of trends is shown in Figure 8.

During April spatial distribution plot of maximum temperature trend indicates an increasing trend all over the Indian subcontinent except for northeast India where the decreasing trend of about −1 °C/100yr to −2 °C/100yr is observed. For Northwest India, an increasing trend of about 1.5 °C/100yr to 3 °C/100yr, over Jammu & Kashmir, Himachal Pradesh, Uttaranchal, and Central India it is about 1.5 °C/100yr to 2 °C/100yr. A higher rate of increasing trend is observed over Peninsular India of the order of about 2 °C/100yr to 3 °C/100yr. In May, the increasing trend is observed over Northeast India of the order of 0.5 °C/100yr to 1.5 °C/100yr and a decreasing trend is noted over Gangetic West Bengal, Jharkhand, Bihar, East, and West Uttar Pradesh. Overall a large west part of India has the highest temperature increase whereas in some regions like Jharkhand and Bengale-Occidental it decreases. An interesting fact to be noted here are the trends of maximum temperature over Northeast India, where negative trend during April and positive during May are observed. And when compared to both the pre monsoon months increasing trend rate is high for April month than of May month. This analysis brings out the possible reasons for the increase of heat waves over Andhra Pradesh and Telangana leading to higher heat wave related human deaths during the last decade.

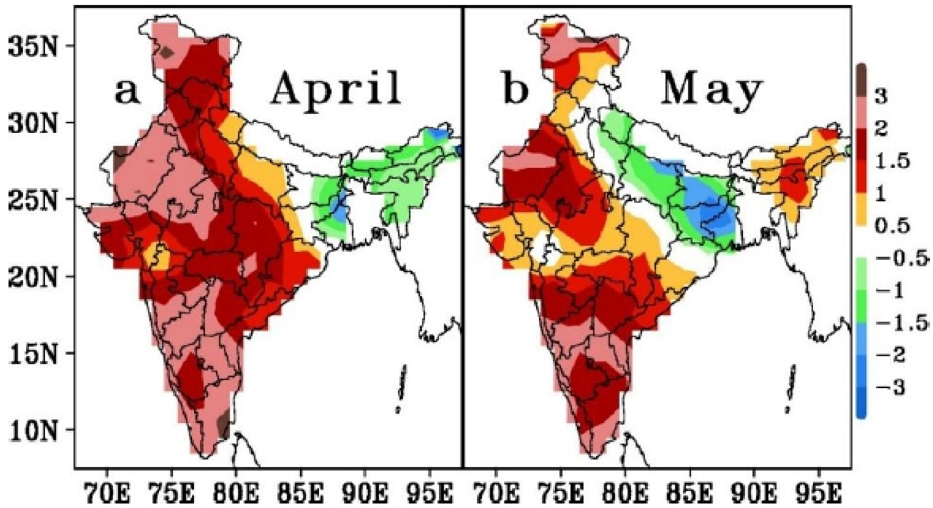


Figure 8. Trends of monthly maximum temperature °C/100yr (during the 69-year period of 1951–2019) for (a) April and (b) May.

3.5.2. Above 42 °C trend

The trends in the frequency of days with maximum temperature above 42 °C are given in Figure 9. During the month of April, trends of days above 42 °C shows an increasing trend over most parts of central and northwestern parts of India with an elongated area/ geographical regional spread of the heat advection diagonally from north western parts of West Rajasthan (29oN) to the northern part of Telangana (19oN) with three concentrated/ isolated pockets. Some parts of Vidarbha and parts of north Telangana show an increase of ~12days/100yr while major parts of the Vidarbha, Marathwada, Telangana, Chhattisgarh, some parts of North Madhya Pradesh, and West Rajasthan show the highest rate of increase of about 6 to 10 days/100yr in April month (Figure 9a).

During May month, the temperature trends with 42 °C days are quite high being a peak month for the hot weather/ pre monsoon season, when compared with April as shown in Figure 9b. A diagonal spread of elongated area of temperature with 42 °C days is high with an increasing trend in the north-western Indian states. A large tract of central parts of Rajasthan (>12) (West Rajasthan and East Rajasthan) are experiencing an increasing trend greater than 12 days/100yr. Major parts of Telangana, parts of Marathwada, and a small part of North Interior Karnataka while the remaining parts of central and northwestern parts of the country, parts of Rayalaseema and Coastal Andhra Pradesh recorded about 6 to 10 days/100yr increase in the frequency of days of above 42 °C. Conspicuously, a negative trend is observed over a major part of Jharkhand, parts of east Uttar Pradesh, parts of Bihar and parts of West Bengal.

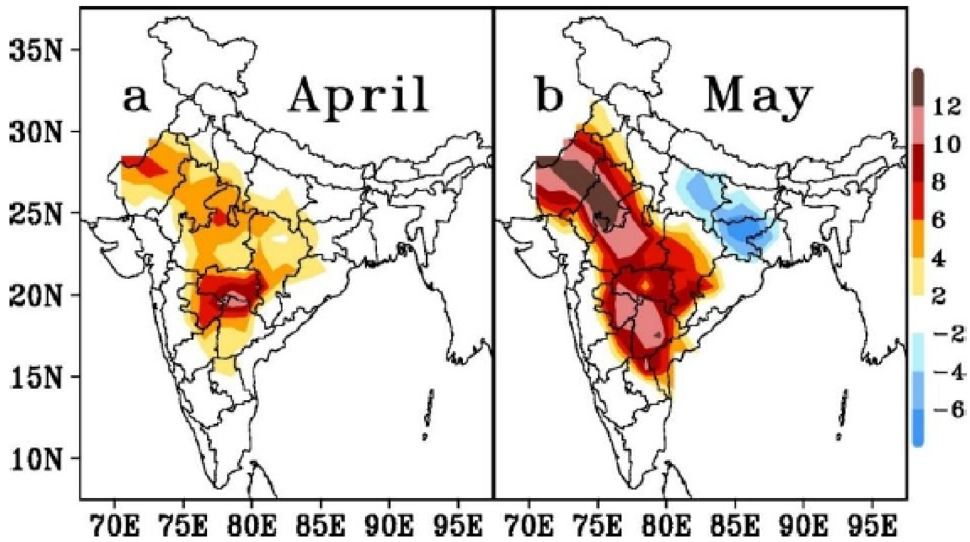


Figure 9. Trends of above 42 °C days/100yr (during the 69-year period of 1951–2019) for (a) April and (b) May.

3.5.3. Heat wave days trend

Spatial coverage of heat wave shows increasing trends in the Indian subcontinent during both the pre-monsoon months i.e., April and May (Figures10a,b). It is observed that the spatial coverage of the increasing trend of the heat waves is more in April than during May. During April month, positive trends of the order of 2–3 days/100yr are observed over central and northern parts of Rajasthan; 1 day/100yr over remaining parts of Rajasthan, northwest and East Madhya Pradesh, parts of Jharkhand, Bihar, East UP, Haryana, and Delhi; of the order of 0.5 days/100yr to 1 days/100yr over the remaining parts of the above states, besides small areas of Coastal Andhra Pradesh and Gujarat.

In May, northwest Rajasthan recorded an increase in the trend of heatwaves of 2–4 days/100yr; remaining parts of Rajasthan, Western parts of Punjab, parts of Haryana, and Coastal Andhra Pradesh show an increase of 1–2 days/100yr of heatwaves; parts of Telangana, west Madhya Pradesh, Gujarat, Telangana, and Rayalaseema show an increase of 0.5 days/100yr to 1.0 days/100yr. A negative trend of heat wave days is observed over the state of Jharkhand, western Bihar, and East Uttar Pradesh.

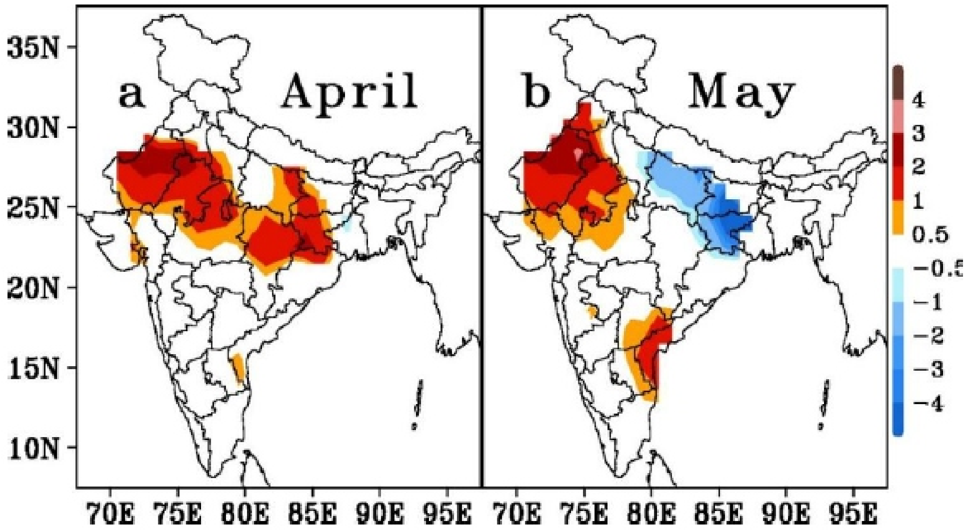


Figure 10. Trends of heat wave days/100yr (during the 69-year period of 1951–2019) for (a) April and (b) May.

3.5.4. Minimum temperature trend

Trends of minimum temperatures were computed for April and May and given in Figure 11. The northern and north-western parts of the country were showing an increasing trend for both the both of April and May and North-Eastern part of the country showing decreasing trend for April and increasing May.

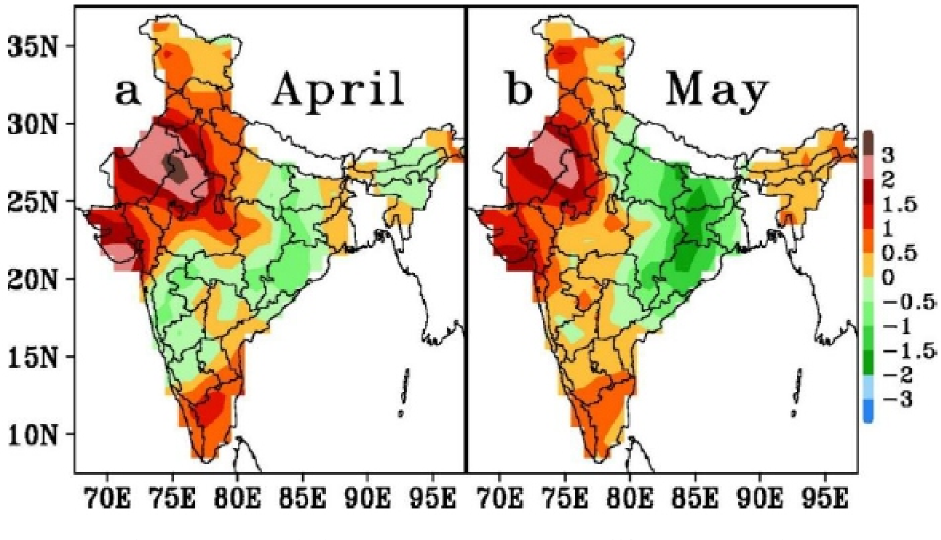


Figure 11. Trends of monthly minimum temperature °C/100yr (during the 69-year period of 1951–2019) for (a) April and (b) May.

3.5.5. Statistical analysis of mean maximum temperatures for T1, T2 and T3 temperature zones and H1, H2 and H3 heat wave zones

Three maximum recorded temperature zones and three heat wave vulnerable regions were identified over the Indian subcontinent [15]. Statistical analysis was carried out for these three temperature zones T1, T2, and T3and heat wave zones H1, H2 and H3 for the period of 69 years i.e., from 1951–2019 as given in Table 1. The areal maximum value of mean maximum temperature during April and May for T1, T2, and T3 are 38.3 °C and 41.3 °C, 39.3 °C and 42.1 °C, and 40.6 °C and 42.5 °C. From this it is clear that T3 temperature zone is occupied with a higher mean maximum temperature during both the pre-monsoon months of April and May with respect to T1 and T2 (Table 2). The temperature zone T1 is occupied with a lower mean maximum temperature for both April and May w.r.t T2 and T3. This gives clear evidence that East Maharashtra temperature zone i.e., T3 has a higher mean maximum temperature than West Rajasthan. Among the three heat wave zones, the third zone i.e., Southeast India is noted to have higher mean maximum temperatures. In April, positive tendencies over T1, T2, and T3 zones are significant at 2.8 °C/100yr, 1.8 °C /100yr and 1.8 °C /100yr (0.01, 0.05 and 0.01 level). During May, the temperatures zone T1 is showing increasing trend (2 °C/100yr) at 0.01 significant level, T2 zone shows a feeble long-term trend (0.5 °C/100yr) and T3 zone shows significant increasing trend (1.2 °C/100yr) at 0.01 significant level. The significant level was tested by using Mann-Kendall test.

For heat wave zones (HW1., HW2 and HW3) during April month, showing positive trend is observed 0.8 (HW1) and 1.7 °C/100yr at 0.1 and 0.01 significant level; HW2 zones showing a decreasing trend at the rate of −1.2 °C/100yr is observed for HW2 at 0.1 significant level. In May, the time series of Mean maximum temperature is showing an increasing trend over HW2 (0.2 °C/100yr) and HW3 (2.0 °C/100yr) zones at 0.1 and 0.01 significant level. The trend values are presented in Table 2.

Table 1. Details of the domain and the meteorological subdivisions corresponding to the identified maximum temperature and heat wave zones.

Temp. Zones	Regions	Heat Wave Zones	Regions
T1 (27.2–29°N; 71.5–74°E)	West Rajasthan	HW1 (27–31.5°N;72.8–78°E)	North India (North Rajasthan, Punjab and Haryana)
T2 (23.8–27.5°N; 75.8–81.2°E)	Northwest Madhya Pradesh (MP), Southwest Uttar Pradesh (UP),	HW2 (21.2–27.2°N;83–87.2°E)	Northeast India (Bihar and Jharkand)
T3 (18.5–21.5°N; 77–81°E)	East Maharashtra	HW3 (12–21°N;79.5–81.2°E)	Southeast India (Andhra Pradesh and Telangana)

Table 2. Statistical Metrics for Temperature zones (T1, T2 and T3) and Heat wave zones (H1, H2 and H3) for the period of 69 years i.e., from 1951–2019.

S.No.	April			May		
	T1	T2	T3	T1	T2	T3
Mean	38.3	39.3	40.6	41.3	42.1	42.5
SD	1.6	1.5	1.3	1.5	1.3	1.3
CV	4.4	4.1	3.3	3.7	3.4	3.2
Trend	2.8(0.01)	1.8(0.05)	1.8(0.01)	2(0.01)	0.5	1.2(0.05)
Above 42 °C						
Mean	3.2	3.9	8.3	13	17.4	20.6
SD	3.7	3.9	6.4	7.2	7.1	7.8
CV	1.9	2.4	3.4	0.8	0.8	2.3
Trend	6.2	4.3	7.3	12.2	4.3	8.8
HW days						
	HW1	HW2	HW3	HW1	HW2	HW3
Mean	38.8	39	40.3	42.1	41.1	42.2
SD	1.7	1.5	1.3	1.6	1.6	1.3
CV	5.6	4.4	3.3	4.7	4.2	3.2
Trend	0.8 (0.1)	−1.2 (0.1)	1.7(0.01)	2.1	0.2(0.1)	2.0(0.01)

4. CONCLUSIONS

The statistical properties of the maximum temperature, above 42 °C days, and heat waves days are evaluated for the Indian subcontinent for the recent 69-year period of 1951–2019.The study assumes importance in the wake of increased heat wave conditions leading to human casualties during the last decade not only over India but all over the world. Spatial distributions of AM, SD, CV, and trends of the monthly maximum temperature field for the two summer months of April and May are evaluated. Important conclusions drawn from the above study are:

(i) Mean maximum temperatures are higher during May, regions higher than 40 °C are observed over central parts of India. A Small region with the highest maximum temperature is located over Vidarbha in central India.

(ii) Cooler regions with temperatures below 30 °C exist over Jammu and Kashmir of north India; west coast and over Assam, Meghalaya, and Nagaland of northeast India.

(iii) SD values were higher over NE India during April when compared to May month. Higher SD values were observed over Andhra Pradesh and Telangana during May month.

(iv) CV distribution shows higher values over the north decreasing towards the south. CV over the south peninsula is higher during May than April.

(v) Heat wave occurrence days computed based on IMD criterion, show higher values over north, northeast and augmented to a greater extent over southeast parts of India are indicating isolated vulnerable regions.

(vi) Climate trends indicate an increasing trend of 1.28 °C/decade over Jammu and Kashmir in North and over the south peninsula and decreasing trends over east central India. The Maximum temperature trend is negative over NE India during April and positive during May month. The increase in spatial extent of heatwaves is observed more in April than in May. The increasing trend over the south peninsula assumes importance because the regions above 38 °C would become more vulnerable to heat wave conditions. Above 42 °C days trend shows negative over Jharkhand, Bihar and East Uttar Pradesh during May month and a higher rate of increase in heat wave days trend is observed during May month over Andhra Pradesh and Telangana.

(vii) A holistic picture of AM, SD, and CV indicates that some selected regions over southeast India with mean temperatures of 38–40 °C have a higher heat wave vulnerability than warmer regions with 40–44 °C located in the northwest.

This study brings out the basic characteristics of the maximum temperatures and the heat wave vulnerability over India during the summer season.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Modeling The Temporal Dynamics of Chlordecone in the Profile of Tropical Polluted Soils as Affected by Land Use Change

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A B S T R A C T

The insecticide chlordecone (CLD) was applied from 1972 to 1993 to banana fields in the French Antilles, which resulted in the long-term pollution of soils and the contamination of crops and water resources. We coupled two biophysical models describing CLD and soil organic carbon (SOC) dynamics to determine the impact of a change of cropping system from banana to vegetable crops on the temporal pattern of CLD content in different soil types, and to assess how this might impact crop contamination and environmental pollution. The results indicated that a change of the cropping system when the CLD content in the topsoil (0–0.3 m) drops below the threshold (1 mg CLD kg−1)established by local authorities to allow the cultivation of vegetablecrops (e.g., cucumber, melon, watermelon, pumpkin), might cause crop contamination due to the presence of relatively high CLD levels in the subsurface layer (i.e., 0.3–0.6 m) of nitisols and ferralsols. The impact of changing the cropping system on the risk of environmental pollution depends on the time of that change, and it is much greater for vegetable crop systems established in the early 1990s following a financial crisis affectingthe banana sector. This is linked to the progressive decline of SOC stocks caused by vegetable crop systems, which reduces CLD retention in the soil and increases CLD leaching. Overall, this study highlights the urgent need to include the monitoring of CLD in the subsurface layer and the dynamics of SOC stocks in current soil testing campaigns carried out on polluted soils in French Antilles.

Keywords: banana; crop contamination; cropping system; French Antilles; leaching; soil organic carbon; sorption; vegetable crops

1. INTRODUCTION

The organochlorine insecticide chlordecone (CLD) was used in French Antilles (Guadeloupe and Martinique) from 1972 to 1993 to control banana weevil (Cosmopolites sordidus), which resulted in diffuse soil pollution that induced environmental damage, crop contamination and severe problems for human health [1]. Chlordecone is a very stable and recalcitrant molecule, with a strong affinity for soil organic matter (SOM) and very low volatility [2]. Cabidoche et al. (2009) were the first to report that the persistence of CLD differs between soil types, ranging from decades for nitisols, centuries for ferralsols to about a millennium for andosols; this is linked to their different mineralogy and SOM contents that markedly affect their sorption properties [3]. Some 30 years after these treatments ended, one third of the agricultural land in French Antilles is still polluted by CLD, and both animal breeding and the cultivation of many crops for human consumption (mainly tuber and root crops) have been banned on these soils [4]. In this context, the Guadeloupe Department of Food, Agriculture and Forestry established three thresholds for soil CLD content in the topsoil of polluted land for the cultivation of vegetables (<1 mg CLD kg−1) and tuber crops (<0.1 mg CLD kg−1), and for animal breeding (<0.003 mg CLD kg−1) [5] that would enable the level of contamination of harvested organs and meat not to exceed the maximum residue limit (MRL) defined by European Union health regulations [6]. At present, only banana, orchards and some vegetable crops such as tomato and pepper can be cultivated in soils containing >1 mg CLD kg−1, because CLD is no longer detected in the harvested fruits [5].

Despite the high stability of CLD, some authors have reported evidence of limited CLD degradation in laboratory experiments under aerobic [7] and anaerobic conditions [8], and its relatively high degradation in the field under anaerobic conditions in a nitisol amended with zero valent iron [1]. Although the CLD content in topsoil had fallen by up to 30% three months after the application of the treatment in the latter study, maintaining anaerobic conditions for several months under real-life soil management by farmers is extremely difficult because of the high water drainage that characterizes the volcanic soils of French Antilles [9]. In a recent study, Cattan et al. (2019) used a model of CLD and metabolite sorption, leaching and degradation to estimate the annual rate of CLD degradation in Martinique soils from data on the CLD and metabolite contents in surface water and groundwater [10]. They found that the estimated annual rate of CLD degradation ranged from 0.02% to 0.15% of the soil CLD stock, which was much less than the rate of CLD leaching. Based on the information available concerning CLD dynamics it can therefore be assumed that leaching is the principal process affecting CLD stocks in the volcanic soils of French Antilles [3,10].

Most studies of soil CLD have only accounted for its dynamics or status in the topsoil layer [3,11,12]. Clostre et al. (2014) analyzed the vertical heterogeneity of CLD levels in six agricultural fields in French Antilles, and observed that although the topsoil layer (e.g., 0–0.3 m) generally contained higher levels than the subsoil layer (e.g., 0.3–0.6 m), the differences between layers were very small for some soils (e.g., 3%), and in one case more CLD was found in the subsoil layer [13]. These authors also noted that within each soil type analyzed (andosols and ferralsols) the CLD content in both layers displayed considerable variability (e.g., the coefficients of variation ranged from 30% to 45%). Such variability may be associated with differences in the total rate of CLD spreading, management of the cropping system and the tillage practices applied by farmers [11]. Cabidoche et al. (2009) also found that the capacity of CLD sorption varies strongly within each soil type, which might differently affect the ratio of CLD content between soil layers since the time of the last CLD application [3]. Taken together, these results suggest that CLD levels in the subsurface layer cannot be predicted directly from observed data on the topsoil layer. Indeed, knowledge of CLD dynamics in the subsurface layer might be crucial when the CLD content in the topsoil approaches the established threshold that permits the cultivation of vegetable crops. This would contribute to reducing the risk of crop contamination for vegetables with a relatively deep root system such as cucumber, pumpkin, melon and watermelon (e.g., 0.6 m depth) [14], which are currently banned on soils with CLD levels >1 mg kg−1 in the 0–0.3 m layer [5].

Another factor involved in the cultivation of vegetable crops is the impact of these cropping systems on the level of soil organic carbon (SOC) stocks, which may affect the sorption properties of the soil. Sierra et al. (2015, 2017) found that although SOC under the current banana and orchards systems are near or at the steady-state, most vegetable cropping systems in French Antilles induce a reduction in SOC stocks at rates that range from 0.5% to 1% per year [15,16]. Sierra et al. (2015) proposed that such differences between systems are mainly due to the frequency and intensity of soil tillage, which is much higher for vegetable crops [15]. It could be hypothesized that the cultivation of vegetable crops after the topsoil reaches the critical threshold may enhance CLD desorption and leaching, and thus increase the risk of environmental pollution. The same could have occurred since 1993 when a financial crisis affecting the banana sector generated a 32% reduction in the area cultivated with this crop that was partially replaced by vegetable and food crops [17]. It is interesting to note that this crisis was not linked to soil pollution by CLD, which was revealed for the first time in the early 2000s [18]. In a recent study, Sabatier et al. (2021) observed that changes in farming practices on polluted soils in French Antilles, including use of the herbicide glyphosate from the late 1990s, induced a rise in CLD levels in marine sediment through the cumulated erosion of contaminated bare soils [19]. To our knowledge, no study has been performed to assess the overall effect of changes in land use and farming practices on CLD dynamics in soil in terms of SOC and CLD interactions. This is necessary to better manage the risk of crop contamination and to prevent any changes to the management of the cropping systems that might cause a further release of soil CLD towards other environmental compartments.

The aim of this work was therefore to determine the impact of changes in land use on the temporal pattern of CLD content in the topsoil and subsurface layers of different soil types in French Antilles (nitisols, ferralsols and andosols). To achieve this, we applied a modeling approach coupling the WISORCH model that describes CLD dissipation [3] and the MorGwanik model that describes SOC dynamics in different soil types and under different cropping systems [15]. Simulations were carried out over the 1972–2045 period, thus including the time of CLD spreading (1972–1993) and the subsequent phase characterized by CLD losses. We analyzed two cropping systems involving banana in monoculture and banana followed by a monoculture of vegetable crops.

2. MATERIAL AND METHODS

2.1. Soils

The soils analyzed during this study are located in Basse-Terre, the main island of the Guadeloupe archipelago (French Antilles) located in the eastern Caribbean (16°05′ N, 61°40′ W). Basse-Terre Island covers 848 km2 and is dominated by a mountain chain oriented northwest to southeast where the crest stands at 1467 m.a.s.l. (La Soufrière volcano). Although the land west of the crest slopes steeply toward the Caribbean Sea (20–30% slopes), the eastern side of the chain slopes more gently toward the Atlantic Ocean (10% slopes). The soils affected by CLD pollution are located in the southern part of the island where banana cultivated as a monoculture is the main cropping system. The soils are nitisols and ferralsols in the lowlands and andosols in the uplands (FAO classification) [15]. The mean air temperature and mean annual rainfall are respectively 25.0 °C and 2200 mm yr−1 in the lowlands, and 23.9 °C and 3800 mm yr−1 in the uplands. Nitisols are rich in halloysite clay (around 50%) and have developed on young ash deposits; the soil pH ranges from 5.0 to 6.5, and the cation exchange capacity from 15 cmol kg−1 to 35 cmol kg−1. Ferralsols are rich in kaolinite (around 35%) and aluminum and iron hydrous oxides (around 40%) and have developed on old ash deposits. The soil pH ranges from 4.5 to 5.5, while their cation exchange capacity ranges from 10 cmol kg−1 to 20 cmol kg−1. Andosols are characterized by high amorphous clay content (around 75%) and have developed on young ash deposits. They are very porous and have high aluminum content; the soil pH ranges from 5.0 to 6.5 and the cation exchange capacity from 30 cmol kg−1 to 50 cmol kg−1. SOC stocks are relatively high in all the soils and vary markedly between soil types (Table 1).

For this study, we selected two soils of each type included in the database generated during the study by Cabidoche et al. (2009) [3]. The soils selected within each type corresponded to plots that had received extreme rates of CLD applications between 1972 and 1993. Some characteristics of the selected soils are presented in Table 1. Note that the level of rainfall varies between soil types and within each type as a function of the microclimate surrounding the plots.

Table 1. Characteristics of the 0–0.3 m layer of the soils analyzed and parameter values for the WISORCH model. Data were taken from the study of Cabidoche et al. (2009) [3].

Soils	Chlordecone spreading Period	Total rate* (kg ha^{-1})	Rainfall** (m yr^{-1})	Bulk density (Mg m^{-3})	SOC stock# (Mg C ha^{-1})	K_{OC}## (m^3 kg^{-1})
Andosol 1	1972–78 & 1982–93	57	6.3	0.40	163	18.03
Andosol 2	1982–93	36	3.3	0.79	83	17.50
Ferralsol 1	1972–93	66	3.6	0.92	58	8.28
Ferralsol 2	1982–87	18	2.8	0.91	60	9.14
Nitisol 1	1972–79 & 1981–93	63	2.5	0.90	51	3.53
Nitisol 2	1972–79 & 1982–87	42	2.5	0.95	40	3.78

*The annual rate was 3 kg ha−1 yr−1 for all analyzed soils.
**Mean value for the 1970–2015 period.
#Initial value for the topsoil layer in 1972.
Partitioning coefficient of chlordecone between SOC and water.

2.2. Model of chlordecone dynamics

We used the WISORCH model proposed by Cabidoche et al. (2009) to assess the dynamics of CLD in the topsoil layer [3]. Taking account of the fact that CLD volatilization is nil [2], and losses of CLD by crop uptake [6] and runoff [10] are negligible when compared to CLD stocks in the soil, this model only considers desorption and leaching as the main soil processes affecting CLD stocks. We extended the original model to account for CLD dynamics in the subsurface layer. We also included a term dealing with CLD degradation by soil microorganisms to assess the potential impact of such process on changes in CLD dynamics, using the approach proposed by Cattan et al (2019) [10].

The basic equations of the model are as follows: for the topsoil layer (i.e., 0–0.3 m in this study)

$$CLD_{n(T)} = [\ (CLD_{n\text{-}1(T)} + S_n) \times (1 - Deg_{(T)})\ ] \times f(Drai_n, K_{OC(T)}, SOC_{n(T)}) \quad (1)$$

for the subsurface layer (i.e., 0.3–0.6 m in this study)

$$CLD_{n(S)} = [\ (CLD_{n\text{-}1(S)} + CLD_{leach,n(T)}) \times (1 - Deg_{(S)})\ ] \times f(Drai_n, K_{OC(S)}, SOC_{n(S)}) \quad (2)$$

with

$$f(Drai_n, K_{OC(L)}, SOC_{(L)}) = \{ \exp [\ (-10 \times Drai_n) \div (K_{OC(L)} \times SOC_{n(L)})\ ]\ \} \quad (3)$$

where the suffixes (T) and (S) indicate respectively the topsoil and subsurface layers, the suffix (L) in Eq 3 indicates (T) or (S), CLDn(L) and CLDn−1(L) (kg CLD ha−1) are the CLD stocks in both layers in years n and n−1, respectively, Sn (kg CLD ha−1) is the CLD spread on the soil surface in year n, Deg(L) (yr−1) is the annual rate of CLD degradation for each layer, Drain (m) is the water drainage in year n, KOC(L) (m3 kg−1) is the partitioning coefficient of CLD between SOC and water for each layer, SOCn(L) (Mg SOC ha−1) is the stock of SOC for each layer in year n, and CLDleach,n(T) (kg CLD ha−1) is the CLD leached from the topsoil and entering the subsurface layer in year n, which is calculated using Eq 1. The factor 10 in Eq 3 arises from the units chosen and allowed us to express CLD stocks in kg ha−1. The exponential term in Eq 3 represents the CLD desorption process which is affected by KOC(L) and SOC(L), and the leaching of the CLD released through water drainage. Note that KOC, as expressed in Eq 3, also depends on soil mineralogy [3,20]. This is further discussed in section 3.1. Eq 2 implies that all the CLD leached from the topsoil layer was a CLD input for the subsurface layer. As mentioned above, this assumption is based on the fact that other CLD losses are negligible in the soils being analyzed. The model considers two types of water drainage: i- forced drainage linked to the stemflow induced by the banana plant, which affects CLD leaching during the five years after the last CLD application; it is calculated as 1.2 × annual rainfall, and ii- after that period, homogeneous drainage estimated according to the water balance [3]. The forced drainage was included in the model to take into account that rainfall redistribution by plant canopy induces strong water drainage at the foot of the banana stem, downstream from the stemflow, which crosses the foot spreading of CLD during the application period (i.e., 1972–1993) [3].

The corresponding CLD gravimetric content (CLDcont, in mg CLD kg^{-1}) of each soil layer was calculated as:

$$\mathrm{CLDcont}_{n(L)} = \mathrm{CLD}_{n(L)} \div (\mathrm{BD}_{(L)} \times \mathrm{Z}_{(L)} \times 10) \qquad (4)$$

where BD(L) (Mg m−3) and Z(L) (m) are respectively the bulk density and thickness of each soil layer. The factor 10 arises from the units chosen and allowed us to express CLDcontn(L) in mg CLD kg−1.

The original WISORCH model accounts for the mechanical dilution of CLD between layers when the depth of soil tillage (e.g., every 5 yr for the banana cropping systems) exceeds the bottom of the topsoil layer [3]. However, it is not clear from the formalisms of the model how the subsurface layer contributes to CLD dilution in the topsoil, and whether SOC dilution between layers is also accounted for. The latter could be important because changes in SOC may affect the sorption properties of the soil (Eq 3). In the present study we did not include any effect of soil tillage on CLD dilution by assuming that tillage only concerned the topsoil layer (i.e., tillage depth equal to 0.3 m). This is further discussed in section 3.1.

2.3. Model of soil organic carbon dynamics

We used the MorGwanik model to assess the impact of changing the cropping system from banana to vegetable crops on the SOC stocks of the topsoil layer, and to set the SOCn(T) value in Eq 1. This model was calibrated and tested using data of the time course of SOC stocks obtained from 253 plots covering most soil types, climates and cropping systems present in the Caribbean, and has been applied successfully to assessing SOC dynamics in many Guadeloupe cropping systems [15,16]. MorGwanik simulates SOC dynamics as a function of annual C inputs and outputs at the plot scale. The basic equation of the model is:

$$\mathrm{SOC}_{n(T)} = \mathrm{SOC}_{n\text{-}1(T)} + (\mathrm{C}_{res,n} \times \mathrm{h}_{res}) + (\mathrm{C}_{ame,n} \times \mathrm{h}_{ame}) - [\mathrm{SOC}_{n\text{-}1(T)} \times (\mathrm{k}_{soil} \times \mathrm{k}_{till})] \qquad (5)$$

where Cres,n (Mg C ha−1) is the C input from crop residues (aboveground and roots) in year n, Came,n (Mg C ha−1) is the C input from organic amendments in year n, hres and hame (unitless) are respectively the humification coefficients of crop residues and organic amendments, ksoil (yr−1) is the SOC mineralization rate constant for each soil type, and ktill (unitless) is the coefficient accounting for the effect of soil tillage on SOC mineralization. Although ksoil is specific to each soil type, ktill is specific to each cropping system (e.g., higher for annual crops than perennial crops). In this study we did not consider the application of organic amendments and the only C input corresponded to crop residues.

As the SOC stock values in the subsurface layer were lacking for the soils analyzed in this study, we used experimental data reported by other authors to set the initial value of SOCn(S) in Eq 2. In this way, SOC stocks in the subsurface layer represented 56% of the SOC stock in the topsoil layer of andosols

[20], 81% of ferralsols [21], and 85% of nitisols [22]. As MorGwanik only accounts for the topsoil layer, we assumed that annual changes in SOC stocks in the subsurface layer corresponded to a fraction of those occurring in the topsoil layer:

$$SOC_{n(S)} = SOC_{n\text{-}1(S)} + 0.1 \times (SOC_{n(T)} - SOC_{n\text{-}1(T)}) \quad (6)$$

where 0.1 is the fraction of the change in the topsoil that was applied to the subsurface layer (i.e., 10%). That value is an average of the changes observed by Poeplau et al. (2011) and Balesdent et al. (2018) in studies dealing with the temporal dynamics of SOC as a function of soil depth [23,24]. Note that SOCn(S) may increase, decrease or remain constant as a function of changes in the topsoil.

2.4. Simulations

A first series of simulations was performed from 1972 to 2045 to assess the effect of soil type on CLD dynamics in the topsoil and subsurface layers. Simulations were made for soils cultivated with a banana monoculture, which corresponds to the more common cropping system observed on polluted soils in French Antilles [10]. This was performed with and without CLD degradation in order to test the impact of this process on changes in CLD content. Data of KOC for the subsurface layer of the analyzed soils was lacking, but the information available indicated that KOC would remain quite stable up to 0.6 m for soils in Guadeloupe [3,20]. We therefore used the same KOC value for both layers in each soil. The same could reasonably be assumed for BD(L) in Eq 4 [21,25]. For the simulations carried out to test the impact of CLD degradation, Deg(T) in Eq 1 was set at 0.0015 yr^{-1} (i.e., 0.15% of the CLD stock), which is the median value reported by Cattan et al. (2019) for natural CLD degradation in the soils of Martinique (French Antilles) [10]. These authors estimated CLD degradation in soil from data on the pollutant contents in surface water and groundwater at the watershed scale, which involved several soil types. Because data of CLD degradation for each soil type is lacking, in the present study we considered the same Deg value for all the soils and layers analyzed. This simplification is further discussed in section 3.2. The parameter values for the WISORCH model are presented in Table 1. The values of soil and banana parameters for the MorGwanik model are presented in Tables S1, S2 and S3 in Appendix A. Both models were initialized with the SOC stocks presented in Table 1.

A second series of simulations was carried out to assess the impact on CLD and SOC dynamics of changing the cropping system from a banana monoculture to vegetable crops. This was performed in two steps. First, we tested the impact of such a change as from the next year when the CLD content was <1mg CLD kg^{-1} in the topsoil layer, which is the maximum CLD level in topsoil established by the Guadeloupe Department of Food, Agriculture and Forestry to permit the cultivation of vegetable crops [5]. In the second step we set the change of cropping system at 1993 for all soils. As mentioned above, the crisis experienced by the banana sector that year induced an increase in the area cultivated with vegetables at the expense banana crops. All these simulations were carried out considering a banana monoculture from 1972 to the year of the change, and then annual vegetable crops up to 2045. The values of soil and vegetable crop parameters for the MorGwanik model are presented in Tables S1 and S2, respectively. Changes in CLD stocks were estimated as for the first simulation series using the parameter values presented in Table 1.

3. RESULTS AND DISCUSSION

3.1. Impact of soil type on CLD dynamics

In this section we present the results obtained excluding CLD degradation.The topsoil layer presented a first phase corresponding to the period of CLD spreading, which was characterized by a rapid increase in CLD levels, followed by a second phase when the CLD content diminished at different rates as a function of the soil type (Figure 1). The changes of CLD content in the subsurface layer depended on the soil type: a continuous increase for andosols (Figure 1a,b) and an increase followed by a decrease for ferralsols and nitisols (Figure 1c–f). The slight temporal fall in CLD levels in the topsoil of andosol 1 (Figure 1a), nitisol 1 (Figure 1e) and nitisol 2 (Figure 1f) during the late 1970s reflected the non-application of CLD during two or three years within this period (Table 1). At the time of the maximum CLD content (e.g., 1987 for ferralsol 2 and nitisol 2, and 1993 for the other soils), the correlation between soil CLD stocks up to 0.6 m and the total CLD rate was small and not significant (i.e., R2 = 0.29, P <0.27). Indeed, at that time soil CLD stocks represented 93% of the CLD applied in andosols, 77% in ferralsols and only 41% in nitisols, which indicates that a large quantity of CLD was lost by leaching below 0.6 m during the period of CLD spreading, and that CLD losses by leaching were markedly dependent on soil type.

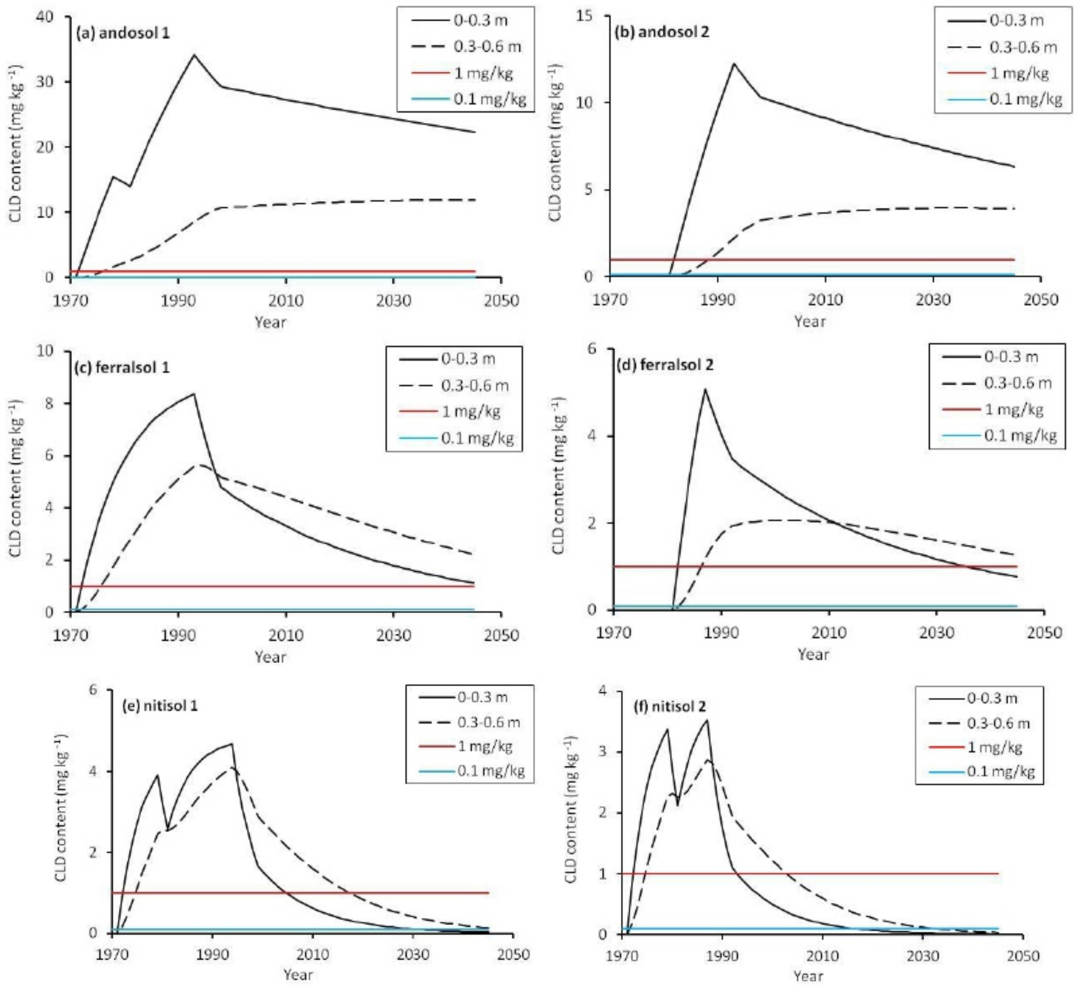

Figure 1. Temporal pattern of chlordecone (CLD) content in the topsoil and subsurface layers of the six soils analyzed under banana monoculture. Horizontal lines at 1 mg kg−1 and 0.1 mg kg−1 indicate the maximum thresholds established by local authorities to allow the cultivation of vegetable and tuber crops, respectively.

During the 1973–1993 period, the rate of CLD leaching varied strongly between soil types: nitisols (average 1.6 kg ha^{-1} yr^{-1}), ferralsols (0.7 kg ha^{-1} yr^{-1}), and andosols (0.2 kg ha^{-1} yr^{-1}) (Figure 2). Differences in CLD content and leaching between soil types can be explained by differences in KOC and SOC stocks, which were the highest in andosols (Table 1) inducing greater CLD sorption and longer residence time in the solid phase of the soil [3]. Cabidoche et al. (2009) had observed that KOC may differ markedly between soils of different types but with similar SOC stocks. This is due to the influence of the different mineralogy of volcanic soils on chlordecone sorption [3]. This was subsequently confirmed by Fernández-Bayo et al. (2013) in a laboratory study [20]. This is particularly true in andosols where the allophane microstructure favors CLD and SOC sequestration [26], thus reducing CLD desorption [27] and SOC mineralization [16]. It is interesting to note that rainfall levels were not the principal factor affecting CLD leaching during the first phase. For example, although CLD leaching was 3.6 times higher for nitisol 1 than for andosol 1 (Figure 2a,e), rainfall was 2.5 times lower for the former (Table 1). Indeed, the high rates of CLD leaching during the 1972–1993 period contributed to the high pollution of rivers, groundwater and crops, as revealed by surveys carried out by the French Department of Health of Martinique in the early 2000s [18].

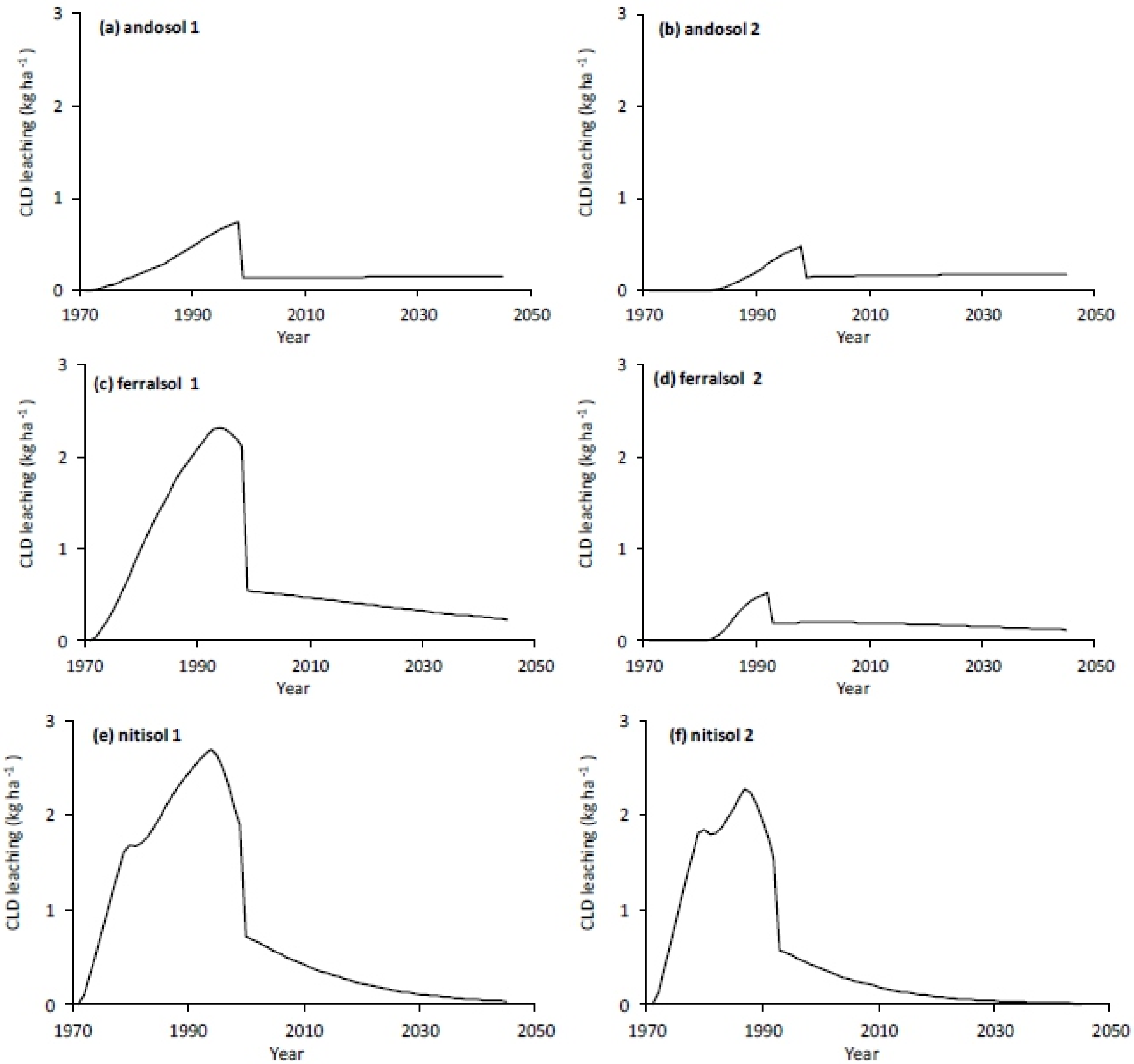

Figure 2. Temporal pattern of the rate of chlordecone leaching below 0.6 m for the six soils analyzed under banana monoculture.

Figure 1 shows that the CLD content in topsoil declined rapidly over the five years after the last application (i.e., until the late 1990s), which was due to both a lack of CLD input and the effects of forced drainage induced by stemflow. During that period, the rate of CLD leaching was relatively high, after which it fell sharply (Figure 2). Because the negative balance between CLD inputs and outputs was less for the subsurface layer of ferralsols and nitisols, the CLD content in these soils was gradually higher in that layer than in the topsoil (Figure 1c–f). For andosols, the balance for the subsurface layer was positive but CLD contents were always higher in the topsoil (Figure 1a,b). Once again, this was linked to the greater CLD retention in the topsoil of andosols that limited downwards CLD transfer. This implies that the proposal made by Cabidoche et al. (2009) [3] concerning long-term CLD pollution of the topsoil of andosols is also applicable to their subsurface layers. The levels of CLD contents and stocks estimated during the present study for the different soil types were close to the experimental data obtained by several authors between 2001 and 2010 [3,11,28] and the data in a GIS database including more recent CLD measurements [29]. In particular, the GIS database shows a regular decrease in CLD stocks in soil from the early 2000s, which is more pronounced for nitisols and ferralsols than for andosols. Moreover, CLD content in some nitisols in the late 2010s is only slightly higher than the threshold of 1 mg CLD kg−1 established by local authorities.

The rates of CLD leaching over the 2020–2040 period differed markedly from those mentioned above for 1973–1993 and decreased in the order: ferralsols (average 0.24 kg ha−1 yr−1) > andosols (0.16 kg ha−1 yr−1) > nitisols (0.08 kg ha−1 yr−1). In other words, although nitisols were the major source of environmental CLD pollution during the period of spreading, at present the main sources would be ferralsols followed by andosols. Indeed, this is linked to CLD stocks remaining in soils after the period of the highest CLD losses. In this sense, Table 2 shows that in 2045, at the end of the period simulated, CLD stocks will account for <1% of the total CLD applied in nitisols, 22% in ferralsols and 70% in andosols. At some sites affected by polluted soils, the shallow water table fluctuates between 0.6 and 3.4 m depending on the soil type and rainfall levels, and appears to be the principal contributor to stream contamination [30]. For this reason our calculations of CLD stocks focused only on the first 0.6 m of soils.

Table 2. Total leaching and soil stock (0–0.6 m) of chlordecone at the end of the simulated period in 2045 for the six soils analyzed under banana monoculture.

Soils	Total leaching		Soil stock	
	kg ha^{-1}	% rate*	kg ha^{-1}	% rate*
Andosol 1	16	28	41	72
Andosol 2	12	33	24	67
Ferralsol 1	57	86	9	14
Ferralsol 2	12	69	6	31
Nitisol 1	63	99	0.4	1
Nitisol 2	42	99	0.1	1

* % of the total rate of chlordecone applied in the 1972–1993 period (see Table 1).

3.2. Impact of natural degradation on CLD losses

The inclusion of an effect of CLD degradation in the model had little effect on the general pattern of soil CLD dynamics described above. Throughout the simulated period, CLD degradation averaged 3 kg CLD ha−1 for andosols, 2 kg CLD ha−1 for ferralsols and 1 kg CLD ha−1 for nitisols. These values represented 7% of total applied amount of CLD for andosols, 4% for ferralsols and 1% for nitisols

(Figure 3). It is clear that in both absolute and relative terms, CLD degradation was dependent on the residence time of CLD in soils, which is directly associated to the sorption capacity of each soil type (e.g., andosols > ferralsols > nitisols). Total CLD leaching at the end of the simulated period only decreased slightly when compared to the results obtained without CLD degradation: 4% for andosols, 3% for ferralsols and 1% for nitisols (data not shown). CLD degradation therefore appears to be sufficient to induce the presence of transformation products in surface water and groundwater [10], but it is insufficient to markedly modify soil CLD stocks. As a consequence, when estimated considering CLD degradation, the temporal patterns of CLD content and leaching were similar to those presented in Figures 1 and 2 for the simulations excluding degradation (data not shown). For andosols in French Antilles, Woignier et al. (2019) reported that allophane microporosity can strongly limit the ability of microorganisms to decompose sequestrated CLD because of mechanical constraints and poor transport properties [27]. It is thus possible that the CLD degradation in andosols calculated during our study overestimated the true degradation in these soils. In the remainder of this paper we will focus on the results obtained excluding CLD degradation.

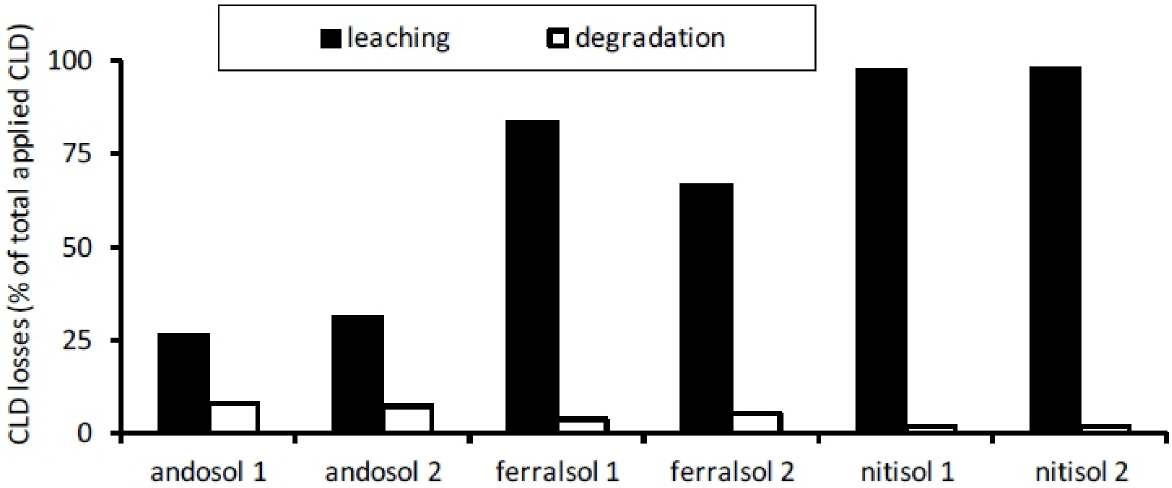

Figure 3. Relative impact of chlordecone degradation on chlordecone losses (0–0.6 m) at the end of the simulated period in 2045 for the six soils analyzed under banana monoculture. Results correspond to the simulations performed considering the effect of chlordecone degradation.

3.3. Relationship between CLD dynamics and the critical thresholds of CLD content

The CLD content in topsoil was equal to the critical threshold of 1 mg CLD kg^{-1} in 1993 for nitisol 2, in 2005 for nitisol 1 and in 2036 for ferralsol 2 (Figure 1). At these dates, CLD content in the subsurface layer were 1.8 mg CLD kg^{-1} for nitisol 2, 2.1 mg CLD kg^{-1} for nitisol 1, and 1.5 mg CLD kg^{-1} for ferralsol 2. The earlier date observed for nitisol 2 reflects its low rate of CLD spreading and the fact that it was last applied in 1987 (Table 1). The CLD content in the topsoil of both andosols and ferralsol 1 did not reach that threshold within the simulated period (Figure 1). Only nitisols reached the second threshold at 0.1 mg CLD kg^{-1} in topsoil (i.e., in 2016 for nitisol 2 and in 2030 for nitisol 1; Figure 1). Some studies carried out in Guadeloupe have shown that the roots of annual crops such as tropical maize can reach a depth of 0.5 m [21]. In addition, it is well known that vegetables such as cucumber, watermelon and pumpkin have a deep rooting system that can reach 0.6 m [14]. These crops are currently banned in soils with >1 mg CLD kg^{-1} in the 0–0.3 m layer [5]. On the other hand, studies performed in French Antilles to assess crop CLD contamination in polluted soils were carried out in the field or under greenhouse conditions using homogenized soils [6,12]. During these studies, only the topsoil was analyzed for CLD content so that any potential contribution of the subsurface layer to crop contamination remained unknown. Our results suggest that the subsurface layer could contribute to attaining crop CLD contents that exceed the MRL when vegetables with deep root systems are cultivated in nitisols and ferralsols just after the topsoil reaches the threshold of 1 mg CLD kg^{-1}. Indeed, further research is necessary to determine the actual CLD content in the subsurface layer of these soils and establish the effects of this layer on crop contamination in the field.

Clostre et al (2014) observed that deeper tillage (i.e., >0.4 m in their study) causes homogenization and dilution of the CLD content in the soil profile, which reduces the differences between the topsoil and subsurface layers [13]. Soil tillage in the more intensive banana systems in French Antilles includes subsoiling at ≥ 0.4 m [31]. Unlike deep plowing and deep mixing, subsoiling aims to reduce soil compaction without turning or mixing soil horizons [32]. Although some parts of the bottom of the topsoil and the top of the subsurface layer may be partially mixed, the precise mixed fraction in each layer is hard to estimate. Therefore, although it is clear that deeper tillage may induce mechanical dilution of CLD and high CLD content in the subsurface layer, as observed by Clostre et al. (2014) [13], such an effect cannot be estimated accurately from the data currently available on the effects of subsoiling. This information could be obtained during future research by estimating the fraction of the mixed layers from data on the CLD and SOC contents before and after subsoiling in soils that differ in terms of their mineralogy and structure.

3.4. Impact of changes of the cropping systems on CLD leaching

As described above, during the first step of this analysis we tested the impact of changes of the cropping systems as from the year after the CLD content was <1mg CLD kg^{-1} in the topsoil layer; this concerned three soils: nitisol 1 from 2006, nitisol 2 from 1994, and ferralsol 2 from 2037. As reported in previous studies performed in French Antilles [15,16], a change in the cropping system may strongly affect SOC stocks, which could modify the sorption properties of the soil and then CLD leaching. The results showed that SOC stocks remained constant during the period under banana monoculture (Figure 4a), thus reflecting that this system is at the steady-state in terms of SOC stocks in French Antilles [15]. This is associated to the fact that C outputs (mineralization) and C inputs (crop residues) converge in the long-term under the same system management (e.g., more than five decades for banana systems in French Antilles) [3,10,15]. Following the change of cropping system, SOC stocks in the topsoil under vegetable crops fell by 1% yr^{-1} on average. This was in line with the data observed in Guadeloupe and reported by Sierra et al. (2017) for monocultures of vegetables when organic amendments were not applied by farmers [16]. Because C inputs and outputs were the same in both nitisols (Table S1 and S2), their SOC stocks converged towards the same new equilibrium at around 36 Mg C ha^{-1} (Figure 4a). High SOC losses under vegetables crops are mainly caused by the high intensity of soil tillage (e.g., up to six tillage operations per year) that enhances SOC mineralization [16]. The ktill parameter in Eq 5 reflects this effect, which is about double with vegetables than with banana systems where tillage is only applied every 5 years [15] (Table S2).

Despite the relatively large fall in SOC stocks under vegetable crops, affecting the sorption properties of the soils, its impact on CLD content and leaching was negligible (data not shown). For example, total CLD leaching at the end of the simulated period rose less than 1% in the three soils when compared to the values obtained with banana monoculture and presented in Table 2. Thus the data presented in Figures 1 and 2 for these soils were not substantially modified by the change of cropping system when CLD content in the topsoil reached the threshold of 1 mg kg^{-1}. Indeed, at that time, CLD stocks were already too small to further affect CLD dynamics and leaching. From these results, it therefore appears that the effect of changing the cropping system when only the topsoil reached the threshold might be more important in terms of crop contamination than environmental pollution.

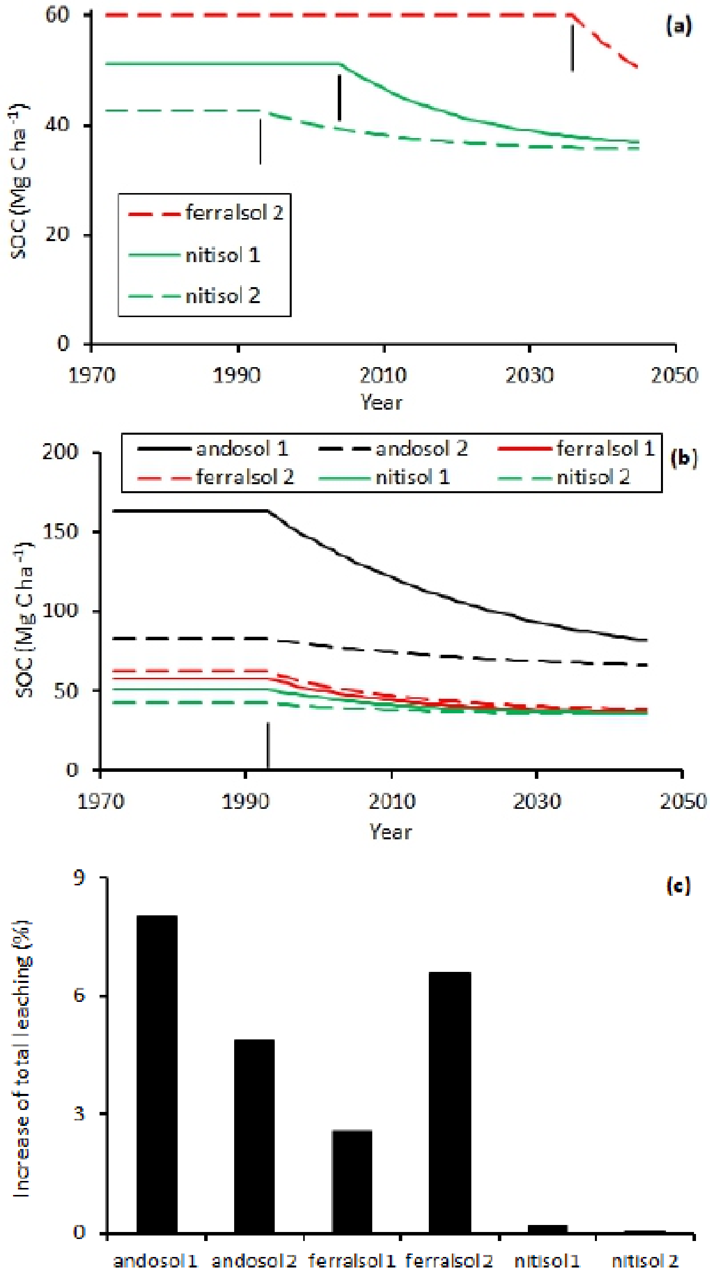

Figure 4. Impact of changing the cropping system from banana monoculture to vegetable crops on (a) and (b) SOC stocks in the 0–0.3 m layer, and (c) the increase in total chlordecone leaching below 0.6 m at the end of the simulated period in 2045. In (a), the change of cropping system was set from the year after the chlordecone content was <1mg kg−1 in the topsoil layer, which only concerned ferralsol 2 (from 2037), nitisol 1 (from 2006) and nitisol 2 (from 1994). In (b) and (c), the change of cropping system was fixed in 1993 for the six soils analyzed. In (c), the increase in chlordecone leaching is related to the values obtained for the banana monoculture system (see Table 2). In (a) and (b), vertical lines indicate the time of the change of cropping system. In (c), the increase in chlordecone leaching is related to the values obtained for the banana monoculture system (see Table 2).

In the second step of this analysis we fixed the change of cropping system in 1993 following the financial crisis experienced by the banana sector. Figure 4b shows that the fall in SOC stocks under vegetable crops was relatively marked in all soils. At the end of the simulated period, SOC losses averaged 32% of the initial SOC stocks. As an index of the effect of SOC changes on CLD dynamics Figure 4c presents the increase in total CLD leaching compared to those estimated under banana monoculture (i.e., Table 2). On average, total leaching increased by 6% in andosols, 5% in ferralsols and 0.1% in nitisols. This increase was negligible in nitisols because their sorption capacity is too small to be further affected by a gradual decline in SOC stocks under vegetable crops. By contrast, the increase in CLD leaching was more notable in andosols and ferralsols with a relatively high sorption capacity and CLD stocks at the time of the change in the cropping system. In these cases, a reduction of CLD sorption through the decrease in SOC enhanced CLD release and leaching. For this reason, the increase in CLD leaching was the highest for andosol 1 (Figure 4c) which had the highest SOC and KOC values (Table 1). These results suggest that the early substitution of banana systems by vegetable crops following the financial crisis might have contributed to increasing environmental pollution and the risk of crop contamination during the past two decades.

Although the partial replacement of banana by vegetables operated in 1993 mainly involved smallholder farmers [17], the land area cultivated with vegetables and food crops on polluted soil has not yet been fully identified. This is a strategic priority for local authorities [4]. Undoubtedly, to perform CLD analyses on each plot in the territory would be a very long and expensive operation. In a recent study, Rochette et al. (2020) proposed a method for the demarcation of CLD-polluted areas based on surface water analyses and the delimitation of hydrological units at the watershed and sub-watershed scales [29]. Such an approach might be helpful to identify plots that should be targeted as a priority during soil testing campaigns, which should also include an analysis of the subsurface layer.

4. CONCLUSION

Coupling the WISORCH model of CLD dynamics and the MorGwanik model accounting for SOC evolution enabled us to highlight the fact that a change of cropping system when the topsoil reaches the threshold for CLD content established by local authorities might increase the risk of crop contamination because of the contribution of the subsurface layer. Although the change in cropping system might not markedly impact CLD fluxes towards other environmental compartments at that time, the impact would much higher for vegetable cropping systems installed in the early 1990s following the financial crisis that affected the banana sector. Under these systems, SOC stocks decline progressively due to intensive soil tillage that reduces the sorption capacity of soils and increases CLD leaching. This would be particularly important for nitisols and ferralsols with a mineralogy inducing a low CLD sorption, which favors CLD leaching.

Our results indicate that in order to reduce the risk of crop contamination and environmental pollution, current soil testing campaigns should include CLD monitoring of not only the topsoil but also of the subsurface layer, paying special attention to temporal changes of SOC stocks in soils cultivated with vegetables. This implies that continuous long-term observations, rather that one-off sampling, are essential to test model predictions for different soil type × cropping system scenarios and improve the accuracy of decision tools at the regional scale. Meanwhile, until decontamination techniques become available, polluted soils should be managed carefully to prevent further CLD and transformation products leaching linked to changes in land use and CLD degradation. To achieve this, farming practices such as reduced soil tillage and the use of organic amendments should be promoted in order to maintain or increase SOC stocks and the sorption properties of polluted soils.

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Conflict of interest

The authors declare no conflicts of interest in this paper.

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Universal Design as Resilient Urban Space Plan Strategy. New Scenarios for Environmental Resources' Sustainable Management

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ABSTRACT

Nowadays, urban framework, stressed by the growing anthropic pressure and its constantly evolving use requests is split by uneven structures, contexts, users and patchy needs, which come out to be inefficient and ineffective in access and management. This fact is directly connected both with morph-functional structure of urban texture and its continuous changing trends. The most important consequences of this situation are some negative effects that produce entropy, mistaken anthropic space uses, ecological networks decrease and most of all a substantial urban life quality reduction connected with mobility problems and non-resilient spaces' use at the different plan scales. These elements make it necessary to restart thinking about environmental resources' sustainable use and management. Universal design comes out to be a useful tool related to urban space planning strategies in terms of resilient choices and actions. Design for all plan approach also represents a good solution for matching people needs to urban environmental quality improvement.

This idea is supported by the experience of a certain case study, that considers universal design application positive effects on open public space plan strategy in Oslo, together with an example of an active use of water to plan a sustainable public space in Rotterdam.

Keywords: universal design; urban context; plan strategy; resilience; environmental resources

1. INTRODUCTION

The ongoing debate over sustainable management of space and environmental resources is most appropriately interpreted in the context of a wide-ranging and diversified body of knowledge, where different concepts and issues related to protection and control of the effects of the emergency generated by climate change in an urban setting take on ever-growing importance in decision-making based on planning, governance and valorization processes on environmental resources and urban spaces, underscored by themes including resilience and anti-fragility. These attributes are the basis for an analysis and identification of solutions for intervening (or not) in the anthropic space of the city.

Nowadays, the organic design of the urban texture, under stress from growing anthropic pressure and its continuous evolution, is fragmented by structures, contexts, users and non-homogenous demands that have a disorganized morpho-functional pattern and inefficient fruition and management. This causes a profound disconnection of urban spaces from the different scales of analysis and planning.

The complexity of city structure comes out to be not-homogenous with disorganized tracts, confused and at times unable to use its own natural, semi-natural and anthropic components to activate and adequately pursue the objectives of quantitative and qualitative sustainable fruition of the various places, at the same time generating functional distortion and environmental impacts at a local level.

For this reason the planning decisions concerning the quantitative use of space clash with the need to recover and add value to the elements used to evaluate the quality of life in an urban context. The urban environment is in constant and rapid transformation, and it tends to forget the role and value of the multiple elements it is made up of. These elements are necessary for the construction, perception and fruition of its landscape, of its distinctive and defining elements, a complex combination of the many characters it is composed of, rich with resources, forms, depths and methods of use in an extremely variable dimension in space and time.

As a result, this deep fracture in the urban system provokes a series of negative effects, including: interruption of the functions of ecological and natural networks, inefficient fruition of space by man, scarce promotion of contexts, entropy, reduction of capillary mobility and management of flows, state of abandonment and environmental degradation leading to a marked reduction in the quality of life in an urban environment, reduced perception of the landscape quality, deviation of socialization practices and participation in the management of local territory by community residents, social exclusion, and tension and conflict in terms of environmental compatibility.

Based on these thoughts, the aim of our research is that to reconsider the methods of analysis and planning in the urban context, as a preferential space for sustainable and resilient anthropic action.

This profound action combines the mainly physical aspect of planning, at the various scales, with the recovery of the identity and role of the urban spaces and their different applies, in a new environmental resources' sustainable use and management dimension.

This work intends to affirm the active role of accessibility as a prerequisite for intervention, as an analysis of feasibility to improve the approach to the idea of anthropic space and its various destinations of use, to activate an integrated system of widespread and environmentally sustainable mobility services. This is combined with the proposal to consider the use of natural open urban areas as the matrix of the project, integrating and completing the goals of inclusion and accessibility, to cope with requests to protect and promote environmental resources in relation to the city.

This idea is supported by the experience of a certain case study that considers universal design application positive effects on resilient open space plan strategy in Oslo, together with an example of an active use of water to plan a sustainable public space in Rotterdam using the water squares.

2. UNIVERSAL DESIGN AS PART OF SPACE COMPONENTS' RELATIONSHIP WITH ANTHROPIC FEATURES

The space to be inhabited defines the complexity of relations in human space in its various modalities of perception, transformation and use; the structure of the place is described in terms of "landscape" and "settlement", and analyzed through the categories of "space" and "character". The two psychological functions implicit in inhabiting a space therefore become "orientation" and "identification". While the space indicates the three-dimensional organization of the elements that make up the place, the "character" denotes the general "atmosphere", which represents the most comprehensive property of any place. This is summarized in the concept of "lived space", meant as knowledge, rediscovery of its earliest meaning and potential, either spontaneously or through the action of man.

Starting from this viewpoint, it is necessary to take into consideration the following approaches: placing the final user as a resource for planning; promoting cultural context and identity; seeking participation in planning; expressing symbolic meaning; creating the conditions for accessibility, advancing differences, studying complexities and analyzing the context(s). In real experience, the definition of accessibility is often taken for granted, or is explicitly connected only to practical rules set forth in regulations.

By contrast, accessibility is a sensitive attribute linked to the relations among different components of the constructed space and the characteristics of the person/user. This attribute is constantly adapted based on the foundational objectives of the project, with a perspective that encompasses a holistic approach to the destination of use of urban space and its resources, at the center of which lies the goal of combining the project with the perspective of the user in terms of efficiency and effectiveness of the access to the spaces, availability and complete, symmetrical circulation of information.

Specifically, we refer to: regulatory accessibility and real accessibility; degrees of accessibility linked to the characteristics of the context(s); accessibility criteria based on: distance, communication (information, legibility, contents, location, lighting, visibility, usability, safety), relation with the city (guaranteed connections), pathways (possible access to a place, an asset, a service; usability of the architectural scale), all items that respond to the fundamental principles set forth in the Universal Design developed by The Center for Universal Design, North Carolina State University in 1997 and related to: Equitable Use; Flexibility in Use; Simple and Intuitive Use; Perceptible Information; Tolerance for Error; Low Physical Effort; Size and Space for Approach and Use.

The concept of accessibility, both in general terms and in relation to the constructed environment – that being the specific topic of this study – is increasingly used and has become commonplace in ordinary language. It is widely used in regulatory and technical texts, substituting the notion of an architectural barrier, which still conveys to those unfamiliar with this field the problem of use and fruition of spaces and services to people with disabilities. To correctly address the issue of its definition, the concept of accessibility cannot be explained without having first delineated an ethical frame of reference. From this standpoint, the foundation is constituted by the 2006 United Nations Convention on the Rights of Persons with Disabilities, endorsed by Italy with Law no. 18, dated 3 March 2009. The importance of the text lies not only in its direct reference to the conditions of persons with disabilities, but above all in the fact that it defined accessibility as a principle in nondiscrimination.

Inaccessibility of spaces, technologies and information is a cause for marginalization and exclusion for people with disabilities, but potentially also for other categories of subjects, who for various reasons and conditions can find themselves in situations where it is objectively/subjectively impossible to overcome barriers. These issues are referred to in the preface of the text very clearly. The Convention acknowledges "... the importance of accessibility to the physical, social, economic and cultural environment, to health and education and to information and communication, in enabling persons with disabilities to fully enjoy all human rights and fundamental freedoms...". Another basic reference regards the definition of Universal Design: "...Universal Design is intended as the design of products, structures, programs and services to be usable by all people, to the greatest extent possible, without the need for adaptation or specialized design...".

Urban spaces and resident communities have serious accessibility issues, and just as often the indicated solutions are designed according to a stereotyped conception for limited niches of potential users, with particular reference to mobility disabilities (It remains emblematic that the dominant signage in urban spaces and buildings to mark attention to accessibility is still the image of a person in a wheelchair.

Elevators, bathrooms, parking lots, just to cite the most common areas in usual planning, are interpreted as accessible in the measure in which they can respond to the needs of people who use a wheelchair.). Nevertheless, the definitive or temporary inability and/or impossibility of a subject to efficiently and effectively use spaces and services encompasses a much wider and diversified group of people, also fragile categories, for example children and the elderly.

In light of the ethical references cited in the UN Convention [1], the most convincing definition of the concept of accessibility is that of Iwarsson and Stahl, based on the concept of the relation between the person and the environment [2].

"… Accessibility is a relative concept, implying that accessibility problems should be expressed as a person-environment relationship. In other words, accessibility is the encounter between the person's or group's functional capacity and the design and demands of the physical environment.

Accessibility refers to compliance with official norms and standards, thus being mainly objective in nature. Whenever using the concept of accessibility, statements must be based upon valid and reliable information gathered in three steps: 1. The personal component (description of functional capacity in the individual or group at target, based on knowledge of human functioning); 2. The environmental component (description of barriers in the environment at target, in relation to the norms and standards available); 3. An analysis juxtaposing the personal component and the environmental component (description of accessibility problems) …".

The relation between person and environment is evident at the moment that the person, with their characteristics and possibilities, "encounters" and often "conflicts with" the requirements and limitations of the environment. The dynamics between person and environment therefore generates a concrete problem of accessibility to the space(s). Therefore, accessibility is not only an attribute of the environment and not only a problem relative to "possibility/ability" of the person.

The definition by Iwarsson and Stahl recalls the objective nature of the problem: the measurability of accessibility. It is not a mere fact of imagining an environment perceived subjectively; the person is to be seen in the perspective of their "functional" profile, which translated into the language used in the International Classification of Functioning, Disability and Health (ICF), can be better defined based on the notion of bodily function and structure (see ICF scientific diagram, Figure 1).

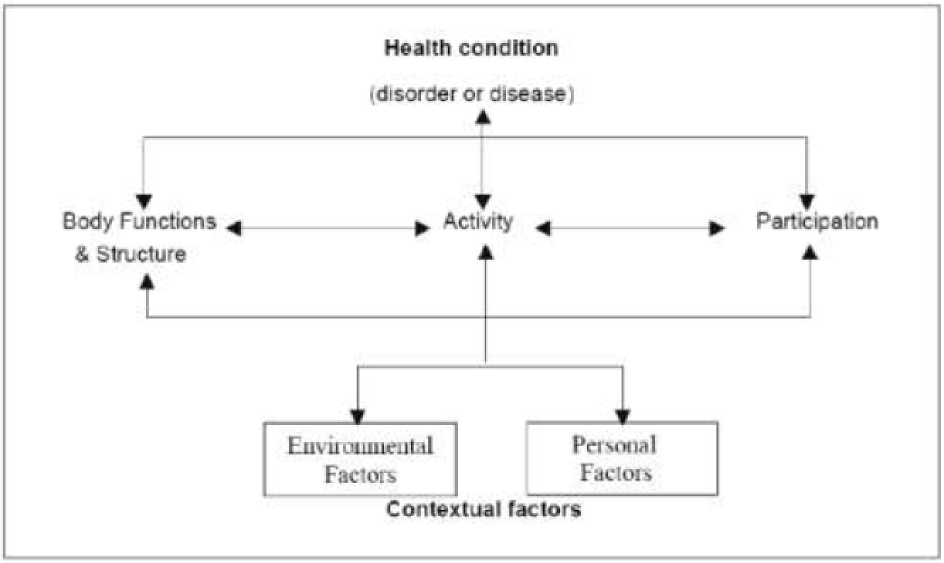

Figure 1. Individual features and built environmental relationship [3].

The environment facing the person reminds of measurable attributes and characteristics, which presumably have the effect of hindering accessibility. This is an open definition that requires a thorough assessment of the functions and structures of the body, including mental and intellectual/cognitive aspects. Accessibility cannot be assessed without considering it in relation to the characteristics of the person. For example, good accessibility must guarantee access to the spaces and services in a simple, independent manner.

Western culture has given preference to the notion of independence up to the point of attributing it with an absolute value, especially in the modern day. Despite this, independence cannot and must not be confused with a final objective, but must be considered as a means for better reaching personal opportunities and objectives (or "capabilities") within a context of complex relations with people, which remains the point of reference for the daily life of each individual.

This concept lends value to the postulate of the Convention, according to which accessibility is a determining factor in non-discrimination, as it guarantees fruition and the possibility of fully implementing the rights of the individual in society.

It is not a given that an environment is suitable for everyone or accessible to everyone, because no one can guarantee that this goal is possible to conceive of or realise. In this sense, accessibility is an open dimension. The law established some characteristics and assumed a fundamental role for public and private subjects. On its own, the desire for accessibility and subsequent planning of the space must exceed standards and regulations, through planning founded on the principles of Universal Design.

Accessibility establishes planning quality: it is impossible to implement the principles of universal design in a mechanical and functional way. The contribution of aesthetics, a fundamental dimension, must also be considered because beauty is an innate need for all people. With regard to this, placing the question of beauty into this equation creates another aspect. During the 1900s – actually probably in the 1800s, the era when the health standards still enacted today were formulated and developed, including assisted residential facilities and institutes – the design culture was directed towards the needs of fragile and disabled subjects, thus generating a health-related form of aesthetics, where the spaces and buildings, furnishings and complementary details had to be different from those made for "standard" people and dictated by health standards [4].

The pursuit of beauty and accessibility should follow a parallel, shared path. Beautiful spaces and things, in addition to being accessible, are the solution that is "best for everyone", underlining also through the architectural language that the pursuit of beauty goes beyond function and destination of use, overcoming discrimination right from the design stage. In this sense, the city can be considered a work of art if it displays its aesthetic dimension, which is inevitably intrinsic to all of its forms of expression. Supporting the idea that beauty should not only be an ideal concept, but instead a practice, implies a clear rethinking of the profile of a city and its development in space.

Discussions on the definition of accessibility highlight that it is not an issue of lacking respect for pertinent laws and regulations, but instead is a theme that must be expanded as far as possible, part of the more general framework of design quality, involving a variety of urban spaces: homes, gardens, public and private buildings, services and technologies.

With regard to this, the preambles and art. 2 of the UN Convention, dedicated to definitions, specify that Universal Design is a tool for rendering constructed space more accessible, attributing a level of importance to design that goes beyond regulatory limitations and rules, nevertheless not excluding their value, since these are obligatory.

But the explicit reference in the UN Convention to Universal Design, characterised by accessibility and usability conditions for all people, has a very dense meaning, because it implements a design model that can no longer be based on the anthropological model of the Vitruvian Man: perfect, abstract and far from reality.

This produced the culture of the "minimum standard", translating into spaces that cannot be used by people with disabilities, which in general do not conform with the needs of the majority of all people.

On the other hand, the Universal Design suggests a pursuit of the "best possible solution(s)" that may respond to most categories of users. This implies a change in perspective in design culture, because it spurs a step from a self-referenced dimension –a type of design that is not attentive to final users, but instead an expression of the designer's sterile personalism, an aphasic design that produces an architecture that does not communicate meaning to the user – towards an externally-referenced design, centred on the needs of the person and enhanced with the components of needs and requirements, able to produce an eloquent type of architecture that communicates recognisable and shared meaning through concrete roots in the context where it is created.

Accessibility therefore is defined as a form of knowledge in design, focused on the individual to respond to their specific usage needs. The resulting architecture presumes a design-oriented thought and action, which moves from the behaviour of users to seek solutions that can prevent discrimination and/or exclusion. These elements, if interpreted in their constructive sense of equality and inclusion, improve the actual and perceived quality of the space with positive, wide-ranging impacts, rendering this architecture the true representation of the society that produced it.

Therefore, inhabiting the space becomes the authentic dimension of anthropic living, where the user finds optimum living conditions and consistent management of the local territory in the medium and long term. This refers to the domestic dimension, to the public space and its relations, to connections generated in the urban texture, which order and direct pathways for fruition of the dense space of the city also from a visual standpoint.

It is in these dynamic dimensions that the very essence of man existing in space becomes apparent, inhabiting the space "to be"; "being in the world"; recognising his role as an active component in the construction and productive use of space, perceiving his own territorial identity, understanding its value, holding its memory, feeling a sense of belonging and safety given by the deep knowledge of the space of daily life [5].

3. MATERIALS AND METHODS: SCHANDORFF SQUARE IN OSLO

The discussion about the re-composition of the urban panorama places architecture in relation to the action of movement, a system of dynamic relations among different spaces in the continuous search for meaning and temporal definition, which constitutes an active method of perception, comprehension and knowledge of the context of the city space. In this sense, movement within the city becomes not only so much a necessity, but an opportunity.

The fragmentation of the urban design is recomposed through the use of accessible planning, which combines the various needs of the users with improvement in the quality of the urban environment. Thus, it becomes a tool that can activate and promote the external positive environmental features to reduce, manage and control the impact, establishing new pathways for moving and living among the different components of the area, creating new scenarios for urban regeneration, relations and resilience between open spaces, in a widespread, inclusive and sustainable way.

Reading to usability on an urban scale, the concept of accessibility has implications that have found adequate expression in the Schandorff Square planning in Oslo, Norway, made by the architects Østengen & Bergo AS [6]

This was an urban renewal project on a public space measuring 2500m2 created in 2009. The design is based on application of universal design criteria, a design model that starts from the needs of people and establishes performance criteria to meet them. This method influences the spatial quality and impacts the fruition of all categories of subjects and potential users.

The result of the intervention highlights an approach to design that produces a marked increase in the quality of the space, as it is not limited to the mere abatement of architectural barriers, but instead reconfigures the framework of the anthropic action through a logic of accessibility permeating the composition (Figure 2).

The result is a solution that implements a profound reformulation of function and dimensions of space, based on the awareness of having transformed limitations into resources for man’s own benefit.

It is a landscape project aimed at restoring collective use in an anthropic context, uniting specific urban renewal elements with re-naturalisation and ecological goals. The square, located in the city centre, is the evolution of a dead-end street, Schandorff, from which the project takes its name, converted by transforming car parking to an urban natural park space.

The square is enclosed by large Oslo buildings: the Trinity Church to the south, a centrally-planned Gothic church with brick facades, the Deichman Library to the east, featuring an imposing row of columns forming the entrance to one side of the square and the home for the ancient sculpture of the Apostle of Greenland, Hans Egede, now enjoying a new enhanced space, in contrast to the previous parking area that overcame the sculpture. The street that comes down Keysersgate opens up a panoramic view to the west.

Figure 2. Schandorff Square view [6].

This project helped to mend the urban construction, managing to reunite the dispersion previously caused by intermittent spaces with impoverished functions, like parking areas, which prevented the possibility to take proper advantage of the space and the surrounding architecture.

The ramp that joins the two opposing urban centres became a compositional hinge-point for the renovated space, unwinding without architectural barriers and enhanced by the axial staircase that connects a residential area of the city to the public library. The ramp becomes an architectural element that generates space articulating into a pathway (Figure 3), an especially significant theme because it carries with it fundamental archetypical values ("…the axis is perhaps the first human manifestation; it is the means of every human act. The toddling child moves along an axis, the man striving in the tempest of life traces for himself an axis. The axis is a line of direction leading to an end. In architecture, you must have a destination for your axis...") [7]

The natural inclination of the area became a design tool, adding value to the pedestrian area and favouring relations, the act of being and moving in space(Overcoming the 7-meter sloping inclination between the two streets generated a solution composed of a connecting curved walkway with a maximum inclination of 6.5%, with insertion of evenly-distanced resting platforms. Along the ramp there are small squares and planted areas that meet one another and alternate in a mesh pattern.) (Figure 4).

In this project the pedestrian area, the quintessential area for a meeting space, is characterised by urban accessibility. Thus, not only does it permit reaching a specific destination, it also suggests a reinterpretation that, in addition to usability, also promotes perception of the space and contributes to rearranging the various parts of the city into a single narrative sequence. This guarantees strong recognition and correct contextualisation of the architectural monuments, which thus communicate the identity of the city.

Figure 3. Plan layout [6].

Figure 4. Plan section [6].

Use of the square is expanded thanks to small gathering areas for socialisation and resting, which open up along the pathway, arranged among the well-kept garden areas, which feature large planted spaces with various flowers and large trees that provide shade alternating with open lawns (Figure 5 a.b.c).

Spaces are furnished with differentiated seating, which creates an interesting feature in terms of accessibility and development of the relational sphere; these seats are many and close together, with benches and revolving chairs with adjustable heights to favour conversation, also fitted with armrests to allow independent movement and seating.

Even the simple path across the square is designed for various categories of potential users, in particular those who may have difficulty managing the incline without resting, like the elderly: just like the seats, the sloping platforms that outline the paths and garden areas are designed for resting, being positioned at heights to serve as back supports.

The materials used are essential, in their resistance and ductility, as well as in their chromatic impact, with warm, pleasant tones. The pathway, with regard to its itinerary, surface and supporting walls, is built in poured concrete with rough surfaces to provide tactile signals for blind and sightimpaired persons. The small gathering areas are paved in light grey granite floors; the areas dedicated to gardens are supported by walls in Corten steel, enclosed by fences with vertical structures that guarantee safety for adults and children, arranged in a pattern to prevent climbing (Figure 6).

In its overall form, the path develops into a cross-over, letting people explore the city like a story. Accessibility plays a fundamental role, as it permits and generates an active appropriation and fruition of spaces, so that they can live up to their full potential, from the creation of opportunities for positive relationship networks, to the creation of a deep feeling of belonging to that place.

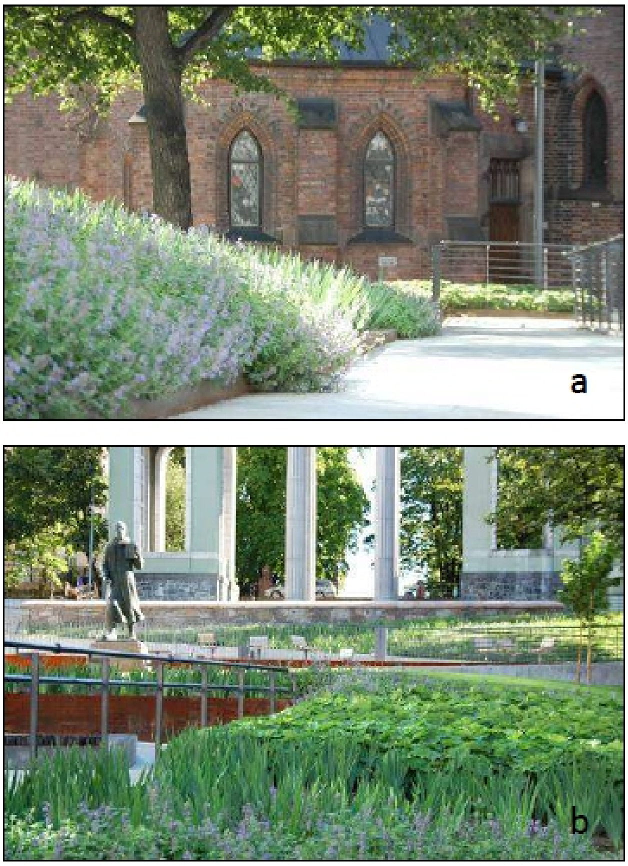

Figure 5. a. Details of the green plan; b. Details of the green plan; c. Details of the green plan [6].

Figure 6. Space reorganisation and assets [6].

4. URBAN SPACE PLAN: SOME METHODOLOGICAL THOUGHTS

4.1. Integrated design

The most direct and practical impact of all this surely falls on an integrated design approach, which unites the well-known issues of composition of space, of urban morphology and the shape of the city, of mobility, management of flows and designing roads and pathways, with the issues of water as an element directly connected to environmental sustainability in the dual dimensions of protection and promotion of resources.

Specifically, this refers to a design approach – at various levels, specific, local, but also territorial – that means to integrate the landscape with mobility and the water mains system, an approach that blends water management with urban planning through multidisciplinary methods, namely: control and management of rainwater in relation to the climate (e.g., in a rainy climate), landscape design principles (study of soil permeability and selection of plants to prevent climatic imbalance), road design criteria (management of different types of flows and multi-modality in relation to speed, geometries of nodes and the impact of flows on the environment, both urban and suburban) within a comprehensive layout, that is the design of public space with its various components.

This line of research, developed through various design experiments, bases its assumptions on four principles:

(1) Rethinking the urban project as an opportunity for a more sustainable water management plan by: transferring rainwater to the aquifer (especially in the urban environment), using differentiated dispensing systems (both urban and suburban), calibrating the most adequate return time in the dimensioning of reservoirs and networks;

(2) Public space planning with more environmentally "convenient" management over time through: separation of white water from black water from the collection point, limitation of use of chemical and mechanical purification systems in favour of more "natural" systems, water treatment systems for fresh rainwater, physical decantation systems, also open air, for screening and storing bulk material in the first stage;

(3) Designing the public space based on hydro-geological safety for the territory (in both urban and suburban areas) through: large underground accumulation basins (water squares) for early emergency response, micro fly-wheel tanks throughout the territory to manage differentiated release over the medium term (both in public and private space), management of waterproofing (use of draining, semi-draining or waterproof surfaces and compensation or hydraulic invariance systems);

(4) Transforming "water limitations and need" into themes for urban and landscaping planning through: expansion tanks and lamination tanks vs. natural areas and parks, underground basins and fly-wheel tanks vs. water squares and rain gardens, banks, holding structures and dams vs. squares, bike paths and urban parks.

This approach provides an opportunity to redirect thinking around new forms of open space, starting from the design of the soil, in particular the types of pavement (waterproof, draining and semidraining), on to the landscape (urban) and vegetation, considered not only as decoration or urban backdrop, but as a "collaborator" with the water project (e.g., rain garden or water squares) and the roadwork project (e.g., as barriers and vegetal filters to absorb traffic emissions), with inevitable consequences on "forms and ways of walking in the city" [8].

The projects developed in this study all revolve around an experimental dimension where new forms of water management through diversification of soil types are tested (to control speed and flow of water), together with use of specific plants for humid or aquatic habitats that can provide better water absorption (with subsequent atmospheric recovery), or mechanically managing rainwater flows, and use of semi-draining pavement to favour differed infiltration over time of rainwater, but with a visual pattern and appearance that integrate with the architectural structures in open public spaces. In short, the entire research study, through experimenting with different materials and functional elements (water and plants), concentrates on the construction of "resilient landscapes".

One of the first specific issues relates to the management of "superficial water" in garden areas and public spaces in the city as a fundamental theme for managing risk and prevention of "catastrophes". Improper water planning can have very serious consequences on the space and its inhabitants: saturation of water networks up to total blockage, flooding and infiltration, and even landslides and collapses.

4.2. Urban design and waterscape

Designing the necessary infrastructures for managing water-related risks follows a discipline founded on statistical determination of critical factors and events and a consolidated practice over time and through history in the territory, spanning farming practices (irrigation) to city defence systems, the need for urban water and sewage systems (collection and flow), and minimum conditions for survival (integrated water system). At times, these infrastructures do not relate to landscape planning practices, especially in the urban environment, since they are always considered "systems", which must collect water (drainage volumes), channel it or hold it (in urban mains networks), and then send it to the aquifer or to other bodies of water (rivers, lakes or sea).

The culture of water system planning often traverses an idea based on risk control, which translates into solutions for limiting danger. Therefore, often the result of vast renovations on water systems in the territory stems from a project that mainly responds to limitations, which are designed to "regularise" and control flooding and the destructive force of water. There was one specific moment in history when the largest landscape and territory projects, from Versailles to Schönbrunn, Caserta to Stupinigi, took advantage of or integrated the limitation of water risk as an opportunity for constructing new local frameworks and original landscape compositions in the geographic scale [9], formulating new inventions, devices and machines for managing water, which today are still a part of common culture for constructing open space areas, because they were then implemented inside the city, such as, for example, grand waterfront projects and riverbed parks in London, Paris and Vienna in the late 1800s, transforming dangerous areas first into work areas and then into areas for leisure activities.

Proper configuration of the landscaping layout, specifically riparian landscaping, in addition to favouring the natural functioning of the water cycle (photosynthesis and return to the atmosphere) and balancing climatic change, also contributes in a significant way to mechanically "retaining" water absorption, therefore providing a mechanism for managing rain events. Moreover, the environmental and natural roles of urban forests, in particular riparian forests, associated with humid lawns, constitute a fundamental element for the correct "systemic" functioning of natural corridors that ensure biodiversity within the city. This is the root of the central role of the water systems in the functional, structural, semantic, management and perceptive construction of open space, starting from the basic components that always have a dual purpose: expansion tanks, lamination basins and water treatment tubs, fly-wheel tanks, holding or overflow banks and barricades, dams, flooded humid fields, canals, and widespread networks. The "basic elements" of the water project become fundamental elements of the project for open space: foundational components for new landscapes, waterways, also spaces that can be used by people if designed for such, like squares, gardens, parks, public spaces, pedestrian paths, sports areas and playgrounds. A valid example of this kind of project is in the city of Rotterdam, Netherlands, reported in Figure 7.

The hypothesis at the base of the waterscape is management of mains water, in particular superficial water flows and drainage, used as the "foundational matrix" of the urban landscape, from which a new vocabulary takes shape, starting with indicators like "permeability", "return time", and the concept of "resilience", arriving at the dimensioning of compartments to derive the figures and forms of open spaces, from modelling of the soil, the arrangement and selection of plants, to the definition and organisation of the functions and fruition levels and accessibility times.

Figure 7. Waterplein Benthemplein - The Watersquare of Rotterdam [10].

Conveying, retaining and then releasing water are therefore the principles used to redesign the urban landscape in an "apparently new" way: gardens, parks, planted areas, squares, parking lots and roads are designed to "collect and retain" significant amounts of water over the span of the year; they are public spaces, but also water system elements. Designed to sporadically absorb significant volumes of water (for example, transforming a square into a fly-wheel tank), in addition to normal operating values, to then release the water, requires significant flexibility in the space and in its function. This does not, however, compromise usability and accessibility. In short, it is a landscape designed specifically to modify its appearance according to the seasonal cycles (the waterscape paradigm). This mutability (another waterscape paradigm) implies significant impacts that must be foreseen and assessed right from the start, because the force of water, if not properly managed and controlled, can wipe the soil and plant life to zero. This cyclical nature is also the motor for environmental biodiversity.

Mutability and cyclical nature are both waterscape paradigms.

5. DISCUSSION AND RESULTS

As noted, the notion of accessibility can theoretically be considered as the result of 5 dimensions:
a) the geographical-territorial dimension, considering the physical distance between user services /resources and the qualitative spatial characteristics of the activity; b) the economic dimension, for which accessibility is assessed in respect to the cost of the transfer; c) the engineering infrastructural

dimension, which considers the type of mobility, promoting transportation as the main the sociological-perceptive dimension, criterion for accessibility; d) the temporal dimension, which defines time as a unit of measurement; e) which relates accessibility to needs, focussing attention on the empathetic aspect instead of the functional aspect [11].

In particular, this latter dimension becomes relevant as in it accessibility is an attribute of individuals rather than of places, overcoming the limits of a perspective that is still too anchored to physical factors, and giving precedence to the relational aspects of the subjects.

The new complexity of forms of the contemporary city requires that accessibility accept and process different meanings and more articulated actions. The profound diversification in ways of using space means that accessibility arranges and directs contacts and the resulting social interactions. From this perspective, accessibility becomes the essence of the urban phenomenon, a principle that favours and interprets the growing lack of uniformity in languages and patterns of the urban texture ("... accessible is that place where each person feels comfortable. A place is accessible when it truly belongs to everyone, a non-discriminating place, non-selective, not alienating...") [12].

In the same way that the environment influences the behaviour of people, anthropic presence and action mark and change the urban environment in a relational pattern involving subjective and collective perception of space. This dynamic establishes an experiential relationship between the user and place, with movement at the centre, for the purpose of recovering its identifying dimension [13].

Mobility, movement within the framework of the city, remains the irrevocable requirement for efficient and effective access and use of places, property and services. "… With the diffused city, instability becomes the dominant character of the urban territory, and in the new contemporary landscape the infrastructures dominate without ever really connecting with it … the mobility of individuals seems to have taken away the genetic code of the city…" [14].

"… The inner transitory urban areas activate new functions, thus becoming strategically linked to and enveloped in the infrastructure, completing it but also acquiring an independent semantic value, designing public space, redefining the relationship between the natural and artificial landscapes. The shared open space therefore becomes a medium between the city and architecture, which tries to be the bearer of an alternative identity, 'a shared space for shared experiences'..." [15].

The methods and speed with which the city transforms in an effort to find an ever unstable balance between usability demands and sustainability of natural, semi-natural and anthropic resources that characterise it require re-thinking the design tools used for the construction of intervention elements that can combine the value of what already exists and the powerful force of what is new.

Mobility and public space become structural systems of the contemporary city; the quality of the urban environment is generated from their synergistic inter-relation, its morpho-functional identity combined with the design of the landscape, as a contribution to the realisation of scenarios in a medium- to long-term temporal dimension marked by resilience and inclusion.

It is this new perspective that influences the most intimate parts of the city, its mutable nature, a knowledgeable experience that is dynamically balanced between memory and experience, making the citizen able to simultaneously perceive their own individual identity and the collective dimension of the urban resident community to which they belong, with the primary objective remaining knowledge.

What emerges is a concrete image of the city, a place in the process of becoming, which possesses and processes the genetic traits of the human being in relation to the attributes of the space offered to him, using the alphabet of accessible design.

Affirming the role of Universal Design in the planning of open space means adopting the perspective of the user; it means abandoning the simplified design scheme based on the standard, categorising final users, where uniformity becomes the norm, disregarding the complexity of reality, the variety of differences, the characteristics of the context, not exalting creative potential.

This approach, which involves a holistic analysis, is made available thanks to accessibility, with the intent of solving the issue of urban planning. It suggests that we face complexity as an opportunity for expanding intervention solutions; that we consider the user as an active resource for design; that we promote the contextual and cultural/symbolic identity of a place; that we implement participatory and inclusive design to prevent forms of discrimination and marginalisation of individuals; that we realise products, environments and services that can be used by everyone, in the most expansive way possible, pursuing optimisation of conditions of availability and use, independently of the initial conditions of the potential user.

The projects developed as part of this waterscape study experimented with the impacts of form/water on new geometries of accessibility/usability of urban public space, starting from a study of habitats, their permeability, the structure of the spatial context and its elements and, in general, the capacity for resilience of the landscape in assessing the role of natural components.

In this sense, the water squares contribute to reducing the critical aspects linked to climate change, favouring the sustainable use of environmental resources, multiplying times and methods for anthropic use of the space in the city.

"... The theme of accessibility involves a series of considerations about the form of spaces and how to arrive at their final planning design. The etymology of the word accessibility – from the Latin accèdere, ad+cedere, walk to/through – incorporates the concept of movement: accessibility, which by definition represents the measure of ease by which something can be reached [16], must be analyzed not only in terms of access to the space, but also in terms of access to its resources. Accessibility is a fundamental privilege for an urban organization and its presence represents a basic index for measuring quality..." [17].

For Lynch, accessibility, together with density and grain, is one of the main characteristics through which the performance of a city can be evaluated [18]. Accessibility is therefore one of the fundamental prerogatives of public space and must be conceived of above all as a requirement for relations among the different spaces in the city.

The deep bond between the environment – understood as the practical, used space – and human intervention, which becomes concrete by way of the implemented planning, forces us to reflect on the essential aspects of architecture to assess accessibility beyond a mere technical/regulatory approach, but in the scenario of dynamic planning, with the goal of maximizing results with regard to function, relation, and service to the user. The profound meaning of inhabiting, in the sense of anthropic use of space, results in identifying with the environment, according to a profile that defines the spatial structures of the place, and in belonging to the same as a choice of existential settling and scheme of use, as a regulatory element of inhabiting [19].

This type of accessibility opens up new scenarios for environmental valorization, an approach that sees accessibility of space and the constructed environment as based on planning knowledge, not simply by eliminating the barriers that hinder widespread and aware fruition by users, but also by maximizing opportunities for efficient and effective use, independently of the potential final user. This requires a radical change in perspective on the urban landscape, not only reformulating ways of seeing, but above all reformulating the action of designing as part of a wider, multidimensional, and multilevel view, to stimulate new ways of actively understanding the urban paradigm.

Nonetheless, in assessing how eco-systemic services help improve the quality of life for urban populations, accessibility combines economic convenience with environmental sustainability as regards intervention decisions involving the anthropic space, generating scenarios marked by resilience, perceivable and assessable positive effects, direct and indirect ecological, environmental, economic and social benefits, cost reduction in the mid to long term.

Therefore, the urban place remains at the centre, intended as the space in which the intervention is implemented and where the relations between people happen, through a design practice that preserves the distinctive natural and semi-natural characteristics, amplifying at the same time the conditions and function of use in an inclusive, accessible, diffused and sustainable way with regard to the environment.

6. CONCLUSIONS

The multidimensional structure of urban space, set in relation to accessible design, highlights its potential for added value in terms of creating an interactive system of services for the city, of high public value, with the objective of reactivating the flows and relations typical of the anthropic space, where the indications relative to the multiple destinations of use contribute to directly and indirectly improve the quality of life for the population in terms of stability and resilience.

From this perspective, the new solutions offered by accessibility – meant as a basic requirement for the design logic, together with a number of detailed micro-interventions guided by regulatory standards – can functionally reconnect the city spaces with its margins, in the scope of rebuilding the urban landscape for the purpose of complete, diffused and sustainable fruition.

What emerges is a new city structure, a new urban landscape that uses modern materials and design elements to regenerate its connective design, creating an accessible and resilient city where the dynamic role of environmental services actively contributes to the quality of life of citizens and a modern inhabiting of the complex spaces and places to come.

The representation of the perceived and inhabited city therefore becomes a dynamic mingling of interventions with a mesh structure that follows, orders and transforms the diversification of its forms of anthropic fruition in time and space, following the evolution of functional relations among its components.

From this perspective comes the effort towards analysis and design derived from modern, open and attentive urban planning; planning that is capable of conveying and interpreting opportunities for participation and complete fruition of attentive, multifaceted living through the identification of intervention tools and elements, in the constant pursuit of a possible balance between the demand for equipped space(s) and the environmental culture [20].

The theoretical/concrete benefit offered by accessibility makes it possible to combine the goals of protection and promotion of environmental resources with economic development over the long term, with particular focus on the parameters of efficiency and economic effectiveness of urban planning choices and territorial management, at the same time marked by the differentiation and synergy of institutions towards optimising the use of spaces and contexts at different levels.

This is urban planning based on resilience, which spurs significant renewal of the contents of projects and plans at every level, in view of making new methodologies available for the acquisition of knowledge that can effectively dialogue with ongoing issues and adequately support assessment of possible scenarios of use, non-use and promotion of anthropic space, without forgetting to account for the quantity and quality of expected direct and indirect benefits, expressed in terms of an increase in collective well-being.

What emerges is an urgent need to rebuild the structural and functional relation between the city and its inhabitants, through the intelligent use of signs and spaces in the urban landscape, using the discontinuity of the elements that it is composed of as an advantage.

The interpretation of the Schandorff Square project clearly expresses how universal design – based on accessibility meant as knowledge, and attentive to promoting the needs of users – has great potential. This is demonstrated in the spatial quality and urban reformulation with surprising results, especially when referred to a design culture that reduces the topic of fruition to the mere elimination of architectural barriers, that considers accessibility to generally be a limitation, if not an outright obstacle to design creativity and expression, a problem that usually hinders architectural aesthetics.

To conclude, the example of water squares in Rotterdam redirects the way of thinking about the urban public space project, which becomes multifunctional if we consider its diverse destinations of use, of being, of moving, of acting, and at the same time contributing to activation of good sustainability practices in the management of environmental resources.

The hope for the future is that projects of this type can pave the way for a new tendency in design culture, with the goal of promoting the quality of the space and the subjects within it., both according to the European Union Green New Deal targets and the United Nations Sustainable Development Goals, connected with a sustainable use of urban space that includes green and blue networks.

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Conflict of interest

The authors declare no conflict of interest.

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Anaerobic Enrichment of Bacillus Alkylbenzene Remedial Consortia from Waste Biomass Melanoid Sources

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A B S T R A C T

Bioremediation of alkylbenzenes, including toluene, ethylbenzene and xylenes, was performed using fermentable aromatic sources and electron mediators by Bacillus cereus 301 in a limited oxygen state. The fermentation of small fermentable aromatic melanoids from cow manure as soluble humus hydrolysates or sugarcane molasses as saccharine, glucose and limited basal medium was compared. Thus, an evaluation model of exponential decline against a control was incorporated for interpretation of remedial data. The significance of the present strategy for constructing multivariant effects of electron donors could be objectively judged by pattern comparison with the short-term data analyzed. Thus, grafted aromatics as methyl- or ethylbenzene require much more microbial reaction time, even with mixed aromatic donors or stronger electron donors such as methanol in the original reduced medium, as indicated in the scatter chart. However, completion of the remedial time was needed by the kinetic simulation, and even low, smooth data were expressed. Among the exponential decay curves indicated, the carbon sources in the mix were favorably expressed. The smooth pattern indicated that fermentations with glucose and molasses showed lower remedial activity than melanoids or the indole series. The vigor increase was better for melanoid carbon in the initial fermentation of 24 h, while molasses increased later at 72 h and was more quenched by amending indole acetic acid (IAA) or indole expression. The molecular interaction of the electron mediator indole acetic acid in most trials indicated a quenched effect on toluene and ethylbenzene degradation, even when mixed with the original reducing medium, but expressed better with molasses in both kinetic simulations and growth effects. Thus, combining electron mediators such as IAA for Bacillus may offer a new degradation route for the metabolite alkylbenzene, which is worth further exploration for environmental aromatic waste remediation and combined restoration strategies.

Keywords: Bacillus; Alkylbenzene; bioremediation; cometabolism; SHH; IAA; anaerobic state; toxic transformation

1. INTRODUCTION

Benzene, toluene, ethylbenzene and xylenes (BTEX) are widely distributed in paint, gasoline additives, chemical foaming, etc., as important blenders for industrial products and bonding agents for architectural materials. As small hydrophobic molecules, they readily diffuse into the lipid layer, which causes chronic deterioration of the respiration system through lipid adhesion and invasion and carcinogenic effects, which cause public health concerns [1]. The recalcitrance toward biological degradation was due to its aromatic ring being prone to damage the cell membrane, bacteria acclimated to secreting surfactant and enzymes, leading to hydrolysis or transformation via hydroxylation, ketonization and carboxylation in the metabolic route, and completion of final ring cleavage via a microbial community involving various respiratory types of microbes [2,3], which have complementary properties via aerobic (+O) or anaerobic (+H) metabolism acclimated to accomplish biological catalytic roles. Nevertheless, the latter has the benefit of saving energy and easily reproducing stable operation in the time of acclimated consortium. In addition to being catalyzed by redox enzymes, aerobic bacteria are prone to secrete extracellular components mediated as surfactants to enhance extraction and exert hydrolytic effects for total petroleum hydrocarbon (TPH) remediation. Parallel results also indicate that over 50% of 3% BTEX could be degraded simultaneously while hydrolyzing hydrocarbons. The integrated activity is stimulated by feeding 1 g/L glucose or 0.5 g/L yeast extract reported as an enrichment carbon source required for Bacillus spp. [4,5], and lithotrophic bacteria from soil could utilize TPH as the sole carbon source [6–8]. Bacillus spp. could be stimulated by cometabolism to stimulate redox enzymes. Additionally, the anaerobic metabolic route proceeded by grafting with long chain acids (for example fumarate into 4-methylbenzylsuccinic acid) was revealed to be the intermediate step for the exchange moiety in the electron-dense position [9–12]. Thus, the transformation step may be slow and hindered despite the fast redox reaction. Thus, the fermentative carbon source of melanoids, molasses, was profoundly enriched in both redox and primary carbon sources for TPH remediation based on their complex properties [13]. Thus, different origins of fermentable melanoids, such as heat and catalytic hydrolysis, have been used as conditioners [14] and further for experimental trials [15,16]. These compounds are worthy of study for their proliferative redox and hydrolysis potential for mediating PAH bioremediation [17–19].

The significance of the intermediate step for anaerobic fermentation [20,21] by modulating molecules–succinyl coA or hydroxylbenzene was indicated as a secondary metabolite that needed stepwise reform of the functional group to trigger bond cleavage. Proliferate aerobics could exert "mono-oxygenase" activity with splitting of water, protonization and formation of ester moieties as phthalate derivatives in the intermediate step during transformation. Previous studies indicated that aromatic mediators could provide suitable chemical forms for alkyl grafting to proceed with reactions [22]. Cometabolites such as methane [23], methanol [24], and organic acids [19] might quickly disrupt bonds hand provide bountiful reducing power for redox reactions, while aromatic derivatives [17] could enlarge their metabolic pool and mediate multiple remediation routes. The significance of implementing aromatic-containing carbon was of interest in possible future development for organic waste recycling and reuse in remediation and restoration.

Yoshikawa et al. 2017 indicated that the conversion of hydroxyl to carbonyl groups is the key pathway for BTEX transformation for further bond cleavage. Presently, we extend our intention to explore biring compounds as mediators for the interaction of metabolism and the switch electron stream to BTEX, especially pertaining to biological waste biomass (e.g., animal feces) or microbe matter (e.g., mushroom waste) for metabolic use [18,19].

Table 1. BTEX bioremediation origin, degrader species, metabolic character and ecological habitat.

Cultivable environment	Microbial origin (Species/Habitat)	Ring transform and cleavage metabolic route (Enzyme pool/route/coupler)	Ref.
Aerobic	*Pseudomonas spp. G*⁻/ Soil	Redox, Mono, Oxygenase, Dehydrogenase, Hydroxylase/Primary metabolite, Catechol	[22]
	Bacillus licheformis/Rumen, *Bacillus cereus* / Low reducing medium/OM, *Bacillus chitinosporus, Bacillus amyloliquefaciens*; (G^+)	Redox, Hydrolase/Low reducing organic matter/Methanol	[16]
	Mycobacterium spp./Ground water & Polluted site	Coculture with primary metabolic degrader/ED	[23]
	Rhodococcus erythropolis/Soil	Redox enzyme/Di-oxo, Di-ketone, Diol, Di-acid	[24]
	Alcanivorax borkumensis G⁻/ Forest soil	Hydrolase, Lipase, Esterase/Carbohydrate, Protein, Oil, Lipid	[25–27]
Facultative anaerobic	*Esherichia coli*, G^-/ Soil	Penicillinase/Secondary metabolite/Fe^{++};Vitamin/Trace	[28]
	Bacillus cereus / Food product	Electron shifting and change oxi-redo state/primary to secondary metabolism	[29,30]
	Citobacter spp./Polluted Soil	Electron shifting and change in oxi-redo state/primary to secondary metabolism/EDM for extension	[31]
Anaerobic	*Sulfate reducer* (G^-)/Wastewater treatment system, *Denitrfier*/Sludge	Fe^{+++}; sulfate/Fumarate, Fe^{+++}; NO^{-3}/succinate; NH_3	[9]
	Bacillus cereus /TPH pollutant soil	Redox, Catalase; Proteinase (Gelatin hydrolysis)/Phenol, Benzoate/Benzyl succinate Synthase; Phenyl succinate, Phenyl fumarate;/*Thiolase*, Phenyl RSCoA;/*Nitrate reduction,*	[32]

In the development of an active consortium that proliferates from soluble humus hydrolysates (SHH) and melanoids, we constructed a facultative anaerobic column system for the acclimation of indigenous consortia from compost communities. Additionally, the bioremedial activity was assessed by analyzing the BTEX concentration until the removal efficiency rose steadily over 85% under daily feed batch operation. After operation for 6 months, isolates of bacteria from acclimated consortia were purified from the sludge under facultative anaerobic conditions and 16S DNA sequencing analysis. NCBI BLAST results showed nearly 100% identical species, and most were related to the genus Bacillus spp. In attempting to judge the physiological role directed by melanoid carbon under a facultative anaerobic state, cometabolic effects were evaluated by melanoid carbon sources, and bicyclic compounds were assessed singly or combined in fermentation. The significance of modulating molecules–as formulated electron donors and mediators (EDMs)–via different grafting properties was thus evaluated. The status of hydrolytic or redox enzymatic targeting for substrate and degradation in the literature is compared in Table 1.

2. MATERIALS AND METHODS

2.1. Acclimation, isolation, and species identification of enriched TEX bioremediation consortia from a coenocyclic column reactor

Polyvinyl alcohol (PVA) porous medium was installed (up to 20% of solution volume) in a 10-L concentric column reactor (Figure 1a) in 3 parallel installations, with a bottom distributed perforating tube for intermittent aerated mixing. The primary mediator soluble humus hydrolysate (SHH) was released from kitchen waste compost (provided by Formosa Corp.) with mixed aromatics containing nutrients in a 1:1 ratio (v/v). The formulation with the final aromatics contained 400 mg/L toluene, ethylbenzene and xylene. Additionally, phenol (PTEX) was added to each substituted benzene for a common intermediate [19,33], and 1 g/L black sugarcane substituent (provided by Formosa Corp.) for daily feeding with a 10-day HRT rate. Phenol was used as a substitute for benzene due to the prohibition of its use over the health risk level. Since the hydroxyl group of phenol was widely used in cometabolic research [33,34] and proved to be an intermediate reactant in aromatic transformation [35], the addition of phenol to the substrate proceeded thereafter. The substrate was fed into the reactor by even distribution at 6-hour intervals 3 times a day to minimize toxic effects. Acclimation proceeded over half a year to stably produce active consortia. Facultative anaerobic conditions were controlled by limited air dissolution by periodic intermittent mixing with filtered air or circulation gas injected by purging for only 3 minutes with a 1/2 hp compressor at a working pressure of 0.4–0.8 kg/M3. After the effluent sample was verified to have a TEX reduction rate over 70% by gas chromatography, the active microbe stage was examined. Sludge consortia were serially diluted and cultured in original reducing medium (R medium) with limited nutrients, minerals, and Fe++/sulfide reducing power. R medium per liter contained 5 mg of boric acid, 0.3 g of magnesium sulfate, 0.5 mg of potassium iodide, 1 g of ammonium sulfate, 5 mg of ferrous sulfate, 0.3 g of sodium sulfide, 0.3 g of dipotassium hydrogen phosphate, 0.5 g of calcium sulfate, 1 mg of zinc sulfate, 0.5 g of brown sugar, 0.5 ml of premixed marine peptone (amino acid melanoid) and 0.25 g of vitamin B [24] per liter of deionized (DI) water. The medium was sterilized under steam pressure at 1.2 kg/CM2 for 20 minutes before adding PTEX carbon and collecting samples at time intervals for analyzing the TEX concentration [11]. Sterilized 10 mM phosphate buffer (pH 7.2) and 0.1% Triton X 100 buffer were prepared for serial dilution (106–108) for single colony isolation to achieve homogeneity under microaerophilic conditions [36,37]. Ten purified isolates were further subjected to 80 mL facultative anaerobic cultivation in 250 mL stainless steel (SUS # 304)-capped serum bottles to avoid air exchange. Two 3-mm tubes with edge neb clamps were assessed by press sealing with butyl rubber. One silicon tube was extended into the medium for ease of sampling with a syringe [38]. Serum bottles were sterilized as usual, and the vitamin and PTEX solution was sterilized by a 0.4 μm filter membrane and amended before inoculation. All experiments were performed in a constant rotary shaker with mixing at 100 rpm (Figure 1).

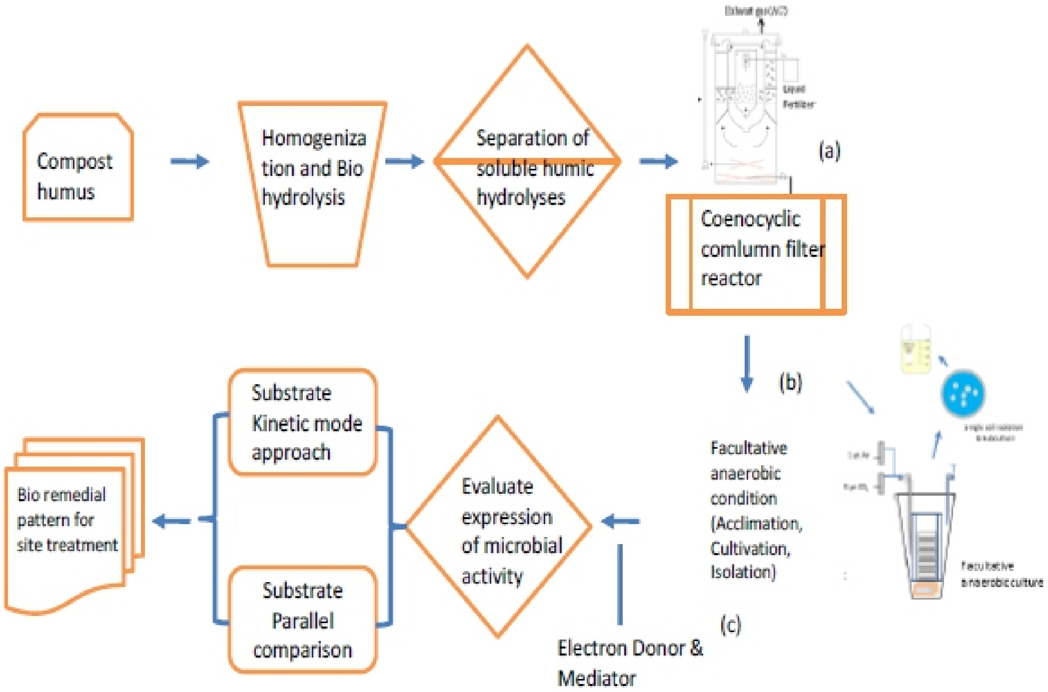

Figure 1. Schematic diagram of the experimental process of airlifting a blanket filter-enriched consortium-facultative anaerobic system for TEX bioremediation. (a) An air lifting blanket filter was installed with 3 layered, concentric columns with PVA foam for enrichment of a consortium of facultative anaerobic microbes (10 L) [39]. (b) Facultative anaerobic conditions involved Petridis cultivation and 250 ml, with limited oxygen status, sampling and analysis by purge and trap GC for TEX. (c) Soluble hydrolyzed humic acid (SHH) was constantly released from kitchen waste compost hydrolysis as an electron donor. Aromatic derivatives, including grafting compounds, were also involved.

Over 15 purified isolates after at least 20 subcultures and a single colony were isolated repeatedly, and these highly homogenous colonies were examined under a microscope (> 600 X) to ensure their homogeneity. Selected isolated plates were analyzed to reveal their proteinase activity, and 16 S DNA gene sequence comparisons were adopted for species identification. Chromosomal DNA primers were synthesized and used in PCR amplification (Bio-Rad 1000 PCR machine, USA) of bacterial genomic DNA (approximately 200 bp). The reaction proceeded in a 25 µL reaction tube using a PCR mix kit according to the procedure for customer service (Protech Technology Enterprise Co., Ltd., Taiwan). Eubacterial primers R16F2n (5'-GAAACGACTGCTAAGACTGG -3') (149-168) and R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG-3') (1373-1397) were used for the reaction. PCR was performed with the initial denaturation step at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 60 s, and final extension at 72 °C for 5 min. The PCR products were further purified by the kit from the Protech Corp, product then sequenced and subjected to BLAST search in the Gene Bank database (NCBI). The results showed the nearest identity specified at the species level, with 100% positivity. Proteinase activity was assayed by ninhydrin colorization with 2% collagen (50 mM pH 7.0 phosphate buffer) as the substrate. Activity was shown when added protein substrate and 0.5 mL 1% ninhydrin solution (in 50 mM, pH 7.0 phosphate buffer), 1.2 mL 50% glycerol (0.2 mL 50 mM, pH 7.0 buffer solution). The reacted solution was placed in hot water (> 70 °C) for heating for 12 minutes, and A570 was measured for evaluation of the amino acid produced. The reaction solute was simultaneously mixed under 120 rpm shaking and reacted for 2 hours, and 200 µL supernatant was collected at 4 °C after centrifugation at 13,000 rpm. The reacted solution visibly turned pink. The standard curve was calibrated by free amino acid (AA) with L-leucine as the end product, and standard solutions of 0, 80, 160, 240, 360 and 400 μg/mL AA were prepared and analyzed as described above. One unit of enzyme activity was defined as 1 μmol/L leucine produced per minute [40]. Protein concentration was determined by a Bio-Rad Protein Assay Kit in 5 minutes. A595 was measured for calibration in a 0–20 μg/mL bovine serum albumin (BSA) linear regression curve to calibrate the concentration. Testing was performed with no cell, enzyme, or substrate as a control.

Electron donors, mediators and facultative anaerobic fermentation. Formic acid and methanol were analyzed as carbon sources. Primary mediators such as SHH were provided from cows by hydrolysis. After composting, acid catalytic (AC) oxidation was performed with 10–30% (w/w) sulfuric catalysts and microelements incorporated with ferrous, magnesium, manganese, molybdenum, and titanium ions (sulfate form, 2–6%) originated in Huang 2019, China Patent CN 106495884A. After steaming and autoclaving at 120 °C and 1.2 kg/CM hydrolysate solids were screened by 12,50 wire mesh and 500 nm PBT pore membranes. Finally, through the finest ultrafiltration (UF) < 5 nm hollow fibers (Spectra / PorMicroFloat-A-Lyzer, Taiwan), a 10 kDa molecule was collected for fermentation. Another primary mediator was molasses, with macroaromatics and carbohydrates. The secondary mediator was physiologically active plant compounds, such as indole and indole acetic acid, formulated for evaluation [41]. Single or double combined additions were tested during the trial and run, and blank series were compared as an uninoculated control - CK; inhibiter formic acid was compared as a control.

Experimental protocol and activity evaluation for TEX bioremediation. Eighty milliliters of original reducing medium had various limited carbon electron donors for maintained vegetative growth. Other extra amended electron donors for analysis included glucose, methanol and formic acid. The primary mediator SHH was molasses. Secondary mediators, including indole and 3-indole triacetic acid, were surveyed for auxiliary mediation effects. Either one with 20 μM chemical amendment with SHH and molasses was followed by stock solution and dosed with A520 0.6, 1 ml added, inoculum suspension supplemented with A605≒1, with a 1% volume ratio. Incubation was set at 26 °C under 100 rpm rotary shaking conditions. Sampling at interval times included initial, 2 hours, 4 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, and 9 days to analyze the volatile components in 3 mL of fermented solution [18]. Data were acquired by concentration pretreated by the VOC purge and trap apparatus (OI-Analytical, 4560, sample concentrator). Individual peak concentrations were analyzed by a gas chromatograph/flame ion detector (Agilent Technology 6850 net GC) [42,43]. The component concentration was calculated according to parallel standard solutions analyzed to build a linear calibration curve and kinetically evaluate the TEX concentration. Degradation efficiency was revealed by subtracting the formulation from the data of the control series. Experimental accessary equipment included microscales, constant-temperature shaker incubators, lamina flow, autoclaves (Tomin, 20 L) and spectrophotometers for cultivation use (Hitachi, model 2000).

2.2. Evaluation of bioremediation activity and growth stability

The bioremediation activity was evaluated by analyzing VOCs using the EPA method. Toluene, ethylbenzene and xylene (meta- and ortho-, with 400 mg/L each), as well phenol, were added to the culture solution by using a Millex-GV Filter, 0.22 μm, 13 mm, sterile syringe filter, and a total test concentration of 1200 mg/L in formulated fermentation R medium broth. Eighty milliliters of sterilized medium including final total concentrations of 1200–1600 mg/L PTEX was used in the test. After 0.8 A605 cell broths were inoculated, and incubation was started, intermittent sampling was performed as scheduled by syringe, and the cells were diluted fivefold in volume. Then, 5 mL broth was injected for VOC analysis by purge and trap equipped GC-FID (hp 6850 with flame ion detector). Calibration analysis of the standard mixture was performed in parallel for calibration and sample evaluation.

For spectrophotometry analyses of the growth status of B. cereus 3-1 under facultative anaerobic conditions, the strains were cultivated in R medium supplemented with different EDMs or synergistic combinations of supplements and incubated as previously described in section 2.1 with capped tubing and sealed bottles. Cell growth under different molecular interaction conditions was intermittently sampled for A605 measurement for analyses of cell density between EDM treatment and the control. The growth status was revealed preliminarily by broth optical density to reveal the growth status.

2.3. Evaluation of bioremediation by kinetic simulation

The transformation activity of active strain 3-1 of TEX data was correlated by global exponential decay curve fitting data of the kinetic simulation mode. To process the batch cultural data, parallel trial experiments were conducted for sampling 3 mL each, ready to be analyzed and calibrated for their concentration. Data were run for simulation by the statistical Sigmaplot Stat software package (Sigmaplot ver. 14, 2019) to evaluate the declining tendency. Differences were considered to be significant by comparison with the variance from the control series. The minute difference between the effect of EDs was judged by exponential decay curve under Pearson's correlation coefficient, $P \leq 0.05$ evaluated. Environmental parameters such as pH, redox potential (ORP), and A605 absorbance were carefully collected for a combined discussion.

3. RESULTS AND DISCUSSION

3.1. Acclimation, isolation and identification of active BTEX remedial bacteria under facultative anaerobic conditions

The screening of BTEX bioremediation isolates from feeding hydrolyzed humus/reducing nutrient solution proceeded from sampling sludge in a 10-L PVA foam reactor. After single colony culture, 9 purified isolates were successfully identified. Strains 3-1, 3-3 and 2-6 were found to have varied properties; 3-1 was found to be a higher gummy substance for mediating adhesion. Additionally, the identification result showed that over 50% of the species were Bacillus spp. B. subtilis, B. cereus or B. thuringiensis are presently designated for use as bioagents against agricultural plant pests and diseases. The evidence for possible incorporation into remediation practice highlights the potential for these bioagents to be further broadly applied for environment remediation through detoxification activity. Surprisingly, 9 isolates were identified, and 5 isolates belonged to the genus Bacillus. B. cereus was more frequently found (Table 2). Thus, B. cereus was the dominant species for starting TEX remediation and worthy of further surveying and revealing for reporting.

Table 2. Bacterial isolates identified from BTEX/melanoid reduced basal medium-enriched consortiaa.

Designation of bacterial isolate	Bacterial strain with 100% similarity in Gene Bank [b]	Specific activity of protease [c] (μmole/mg protein/min)
2-3	*Bacillus subtilis* sH-3	0.244
2-6	*Lysimnibacillusmaroides* sCS-26	0.261
3-1	*Bacillus cereus* s133	0.222
3-3	*Bacillus cereus* s125	0.252
3-5	*Bacillus thuringiensis* sS3-3	0.222
3-7	*Bacillus cereus* nZ2-1	0.197

Note: a 10-L PVA foam biofilter reactor was acclimated by feeding SHH/R medium over 6 months. Then, the reactor consortium was sampled for culture and isolation. Microbe samples were serially diluted by a factor of 106–107. Isolation was manipulated with R medium, and subculture was repeated until the medium was purified microscopically. b Bacterial identification was performed by over amplification with primers and 16S rDNA fragments, and the amplified products were sequenced by BLAST NCBI for species identification. All species named were 100% positive for species identification. c Proteinase activity was indicated by the ninhydrin method. Specific activity was calibrated by a leucine or BSA standard curve; protein concentration was determined by Coomassie blue reagent (Bio-Rad), calibration by BSA (Sigma).

3.2. Effect of electron-rich donors with melanoid mediators on the bioremediation of TEX by B. cereus 3-1.

The remedial activity of strain 3-1 in the original reducing medium was determined with an in-350 mV ox redo potential donated by Fe++ reducing power. After as short as 4 hours of incubation, TEX levels all declined exponentially (Figure 2A,B). Toluene was most reactive when SHH was added with methanol, less reactive with molasses, and did not improve with methanol amendment. The increase in remedial activity with SHH may result from an increase in reducing power due to the small molecular size, and additional functional groups from the surface of SHH might be suggested. The decreased concentration from the background to the control (no inoculation series) explained the adsorption effect against the bioremediation reaction (Figure 2B,D). The remedial reaction indicated a gradual curve as the latter growth curve pattern varied. As shown in Figure 2A, after 3 hours of incubation, the formulation as molasses reacted better than SHH and exhibited significant lowering of toluene concentration by a suitable combination with methanol for a strong electron donation effect (Figure 2B,C). The earlier remediation effect by molasses was better for reaction with multiside chains for xylene and was easily elicited in a reduced state. Overall, the SHH series expressed higher transformation activity and showed an exponential decay curve (Figure 2B,D,E). Second, among the TEX compounds, xylene had the lowest solubility, while it was reactive for most carbon sources provided in the bioremediation removal reaction (Figure 2E,F). Therefore, high removal throughout the cultivation responded with solubility, while the mediation state was highly positive and interacted on the SHH surface.

The survey of melanoid sugar molasses as a mediator of hydrophilicity via a surface hydroxyl moiety. Constituents including carbohydrates and adsorption spectra of UV-VIS of soluble melanoids showed a broad band of brown organic matter. However, the reaction was ineffective and even quenched with the addition of methanol as a stronger electron donor. Additionally, molasses proved to be inert to the remedial reaction. The unexpected enhanced reaction was succeeded by combining SHH with methanol, which was more effective in donating the electron potential needed. These results led to the suggestion that molasses was not suitable for bioremediation or molecular orientation/mediation, even though the molasses had already been used in industrial production. In comparison, SHH was more suitable for reaction. The possible favorable properties of SHH relied on its more homogenous molecular size, which facilitated the induction of biological catalytic reactions. In contrast, dual methyl moiety grafting for xylene was effective for subsequent remedial reaction (Figure 2C,E,F).

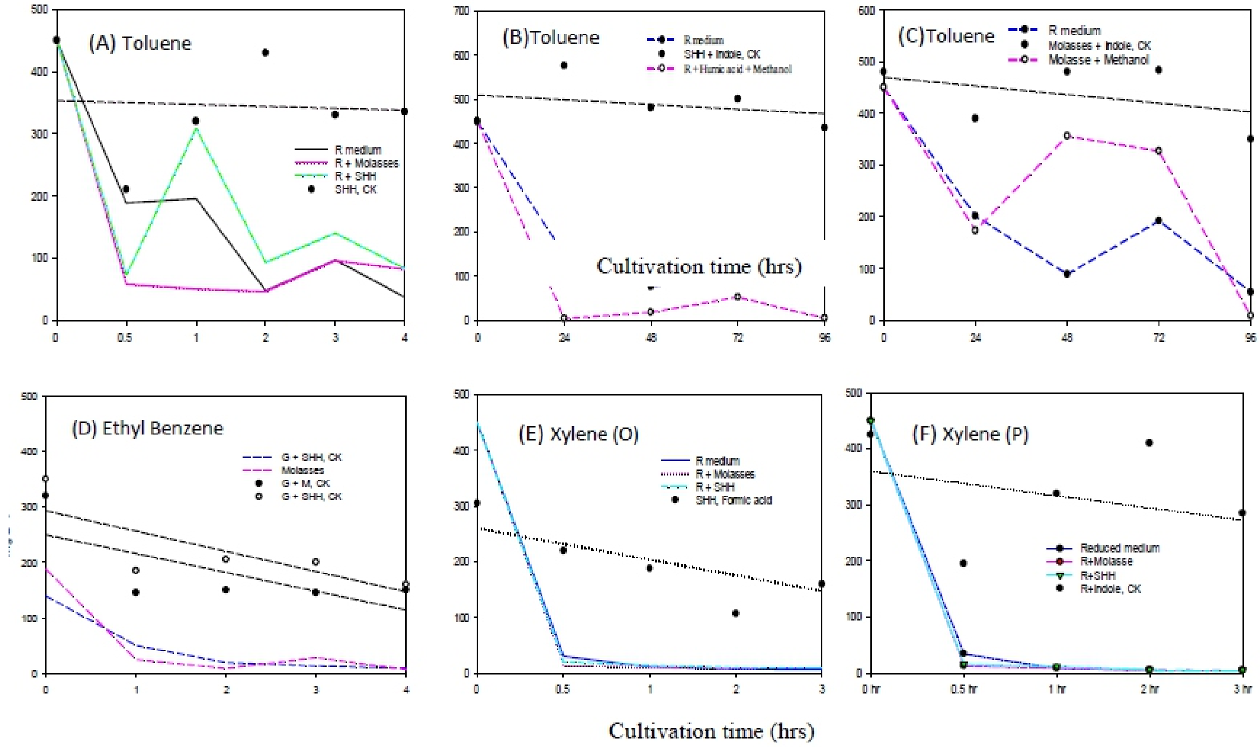

Figure 2. Effects of electron donors on the bioremediation of toluene and xylene by Bacillus cereus 3-1. A) Effects of molasses and SHH amended in basal media with toluene. B) and C) Effect of the addition of methanol with SHH or molasses amended in basal medium with toluene. D) Effects of molasses and SHH amended in basal medium with ethylbenzene. E) and F) Effects of molasses and SHH amended in basal media with xylene.

3.3. Fermentation of TEX with a melanoid carbon source and mediators by Bacillus cereus 3-1.

Since BTEX bioremediation was not effectively achieved via Pseudomonas spp. in an anaerobic state, we began to postulate that intercalation by TCA acid had a limiting factor, in which an epoxide intermediate might be involved in anaerobic transformation. Transforming via hydroxylation by SCOA (S-thiolation by coenzyme A) reducing power initiated cleavage aromatic bonding [9]. The proposed mechanism presently found that a donor with electron reducing power such as methanol and glucose could meet the demand for anaerobic degradation. Figure 3A,B shows that glucose added individually or combined with molasses performed an immense biological remediation reaction, which indicates that hydrocarbons in brown material facilitated higher reactivity extending to 210 hours, which meant that these aromatic carbon sources donate a longer-term reducing power than carbohydrates. Furthermore, an equally amended primary mediator–SHH–with a secondary mediator–indole - neglected its much higher adsorption background than molasses in the uninoculated control (Figure 3C,D). It was not indicative shown that indole was reactive relative to the control, with high adsorbing and decreasing TEX, but might be reactive through an aromatic ring and small grafted molecule - SHH. The control pattern also showed that decreasing TEX initially led to higher growth, which meant that SHH could elicit reactive and possible mediation through multifunctional groups other than molasses. In further consideration of the survey, secondary mediators, such as grafting alkyl chains (IAAs), were analyzed.

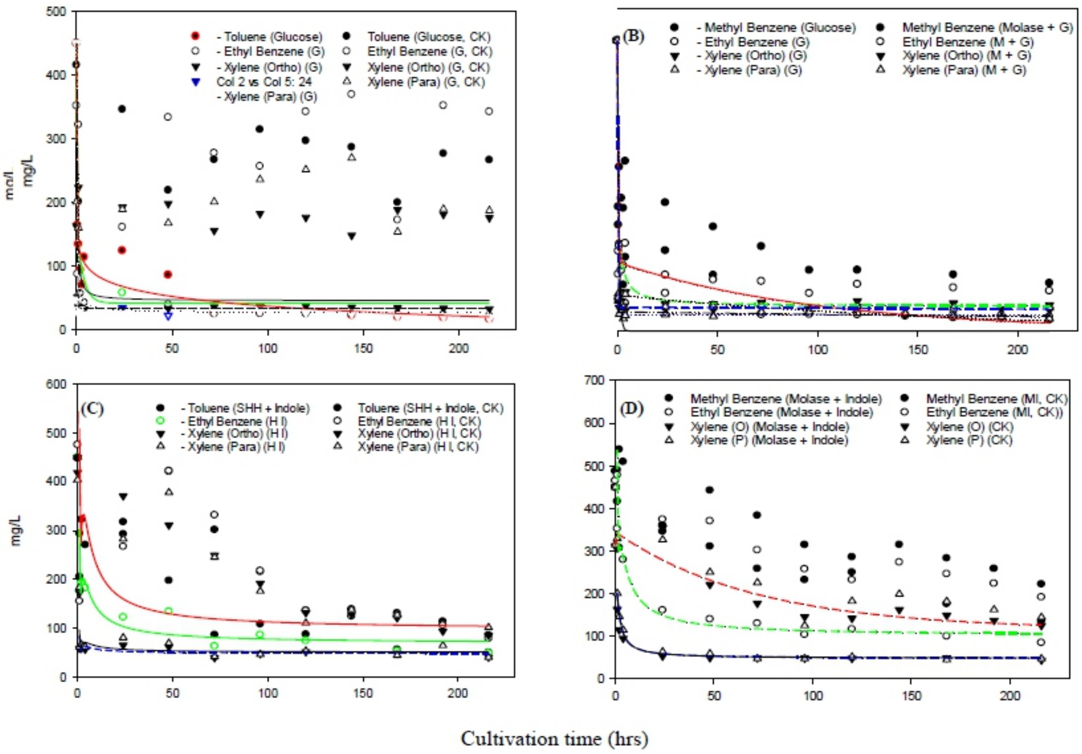

Figure 3. Effects of electron donors and mediators on TEX bioremediation by Bacillus cereus 3-1. A) 20 mM glucose (G), without bacterial inoculum as CK. B) 20 mM glucose (G) with or without molasses (M) (A440=0.25). C) SHH (A440=0.20) + 20 mM indole (I), without bacterial inoculum as CK. D) Molasses (M) (A440=0.25)+20 mM indole (I), without bacterial inoculum as CK.

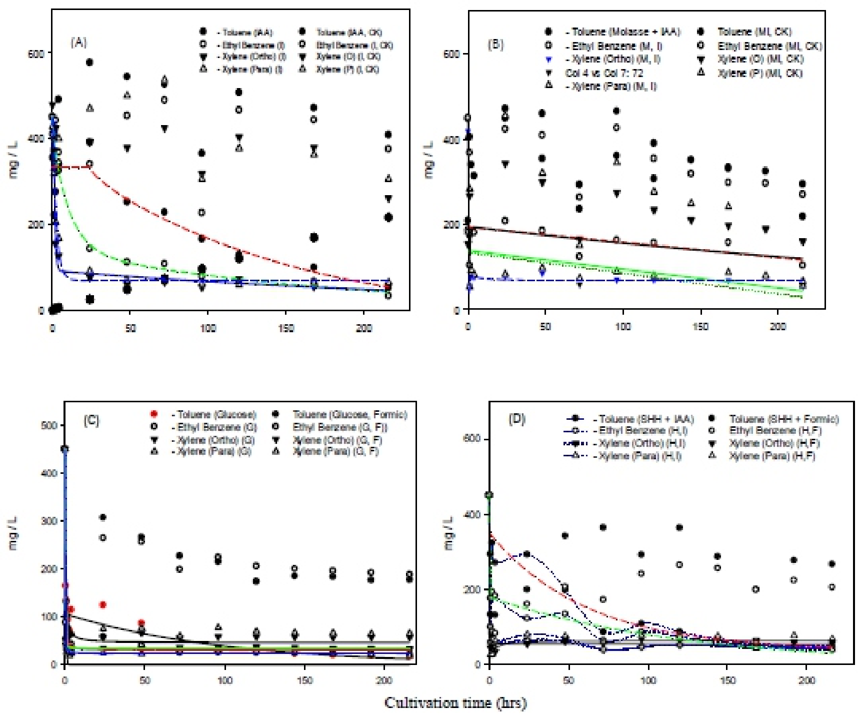

Figure 4. Effects of electron donors and grafting mediators on TEX bioremediation by Bacillus cereus 3-1. A) 20 mM IAA (I), without bacterial inoculum as CK. B) Molasses (M) (A440=0.25)+20 mM IAA (I), without bacterial inoculum as CK. C) 20 mM glucose (G) + 20 mM formate (F). D) Soluble hydrolyzed humus (SHH) (A440=0.20) + 20 mM IAA (I), SHH + formic acid (F).

The grafting of aromatic molecules has many characteristics that induce aromatic transformation. First, as IAA has an activating role in regulating the elongation of carbohydrate polymers, IAA has a more grafted acetyl moiety in addition to ring structures for interaction, a key characteristic for physiological stimulation of bacterial metabolites [7]. Second, the richness of electron flux in the ring structure provided antioxidative and reducing power, such as pigments, e.g., chlorophyll and anthocyanin, while oxygen bonding in the carbohydrate ring blocked electron motility and inactivated the terminal alkyl hydroxyl group to terminate the oriented reaction. Thus, the IAA structure increased electron movement toward the ring structure and initiated nucleophilic attachment via free radical formation, which was reasonably proposed. Third, an alkyl hydroxyl structure was generated as an intermediate step by dissipation of electrons and facilitated aromatic bioremedial reactions. IAA accumulated in the rhizosphere from microorganisms (including pathogenic bacteria) by phytoremedial plants. The bioremediation activity among the participants was postulated to be elicited by these mediators [36,44,45]. The ecological system has a delicate balance between plant species and microbes, and Trichoderma spp. Biocontrol fungi could promote plant growth via IAA and its derivatives, and environmental conditions (e.g., water content and aeration/energy source) were intimately associated with bioremedial conditions in which the oxygen supply was involved in those metabolic processes. Therefore, microbes produced via facultative anaerobic fermentation with reducing carbon sources have been studied for remediation [46].

The present results supported that IAA could participate in remedial reactions by facilitating better bacterial fermentative growth. Furthermore, the combined melanoid addition-mediated synergistic effect was better with SHH than with molasses alone (Figure 4A,B,D). Since molasses resulted in higher growth by providing hydrocarbon content, glucose or fructose, which favored vegetative growth and synergistic remedial reaction, was delayed until 140 hours of incubation (Figure 4C). These results indicate that Isolate 3-1 shifts vegetative growth to effective bioremediation, degrades TEX by slow fermentation, releases reducing power from electron donors through grafted short-chain interactions and further mediates polar group formation. The synergistic reaction with SHH + IAA started early at 4 hours of cultivation and stably increased even though bacterial growth was limited but still physiologically active for remedial activity (Figure 4D). Therefore, the electron reducing power in the microniche-catalyzed, ring structure derivatives started to react via electrophilic substitution driven by the electron potential of the alkyl substituent. Overall, SHH improved the remediation activity over toluene and ethylbenzene, as shown by the kinetic - exponential decay curvature difference. The extended growth curve indicates synergistic reaction with IAA and methanol, even under limited vegetative growth.

3.4. TEX bioremediation with anaerobic growth of Bacillus cereus 3-1.

Both amendment with indole and IAA could perceivably extend the bioremediation reaction time. The logarithmic growth was influenced by linearizing or lowering the remedial tendency by lengthening the cultivation period. The possible phenomena become linear growth through changing the physiology of vegetative cells according to the bioremediation mechanism. The electron donor and mediator did not efficiently react as high reducing power with glucose and methanol but slowly decomposed in the remediation process after 150 hours of incubation. The significance of SHH played a synergistic role over the primary decomposer, and a transformation reaction was suggested. These results also indicated that support for secondary mediators such as indole or IAA could partially provide suitable extra reducing power in aromatic transformations. Figure 5A shows that SHH and molasses had an opposite switching effect toward indole into TEX utilization and transformation metabolism. Molasses showed slower bacterial growth until late at 100 hours and diverted into a logarithmic curve again, while combined SHH with IAA maintained the initial 60-hour growth but declined sooner than molasses (Figure 6A), so more SHH might be needed and expected. At the beginning of the latent phase in the SHH series, growth could still be observed in the early latent stage as a single ingredient. As the experimental TEX concentration reached a maximum, notably, an adaptive strategy combining electron donors and mediators, such as combined SHH and IAA, showed a strong response. As in the initial 2 hours after inoculation, a drop in cell concentration led to a possible discriminative response to bioremediation. After incubation for 4 hours, the bacterial cell concentration steadily increased, indicating that bacterial growth responded to these additives, especially toward growing active cells between 48 and 72 hours, and TEX was thus bioremediated (Figure 5B).

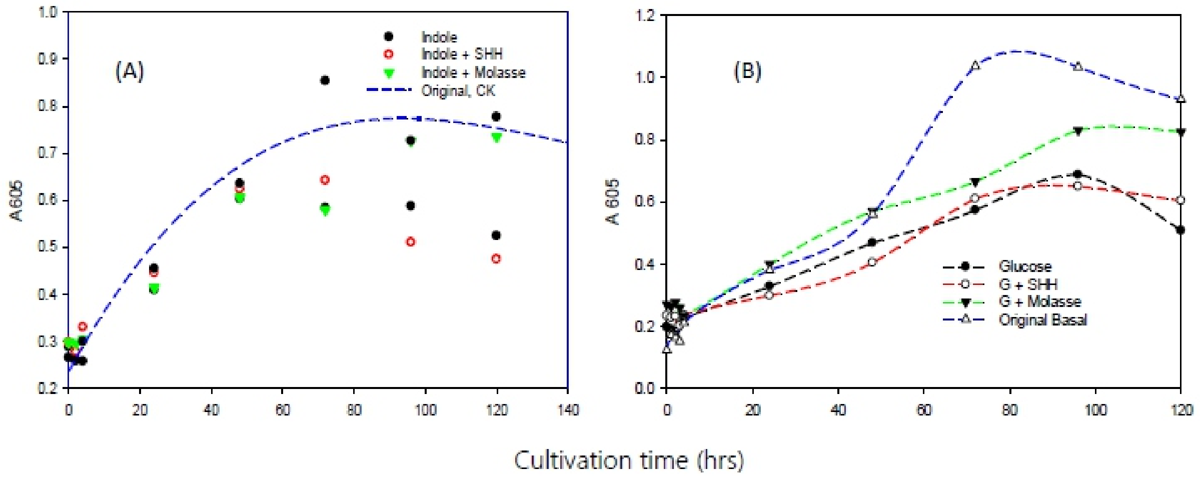

Figure 5. Effects of electron donors and grafting mediators on the growth of the active TEX degradation strain Bacillus cereus 3-1. A) 20 mM indole, SHH (A440=0.20), molasses (M) (A440=0.25). B) 20 mM glucose (G).

The decoction of TEX by purge-trap analysis succeeded in revealing that the amount of aromatics had declined during the bioremediation with MA, soluble SHH and bicyclic ring compounds - IAA via a hydrophilic carboxyl group and triggered high activity even after 76 hours and continuously maintained them to 9 days (216 h) (Figure 6B), as compared with the check - no inoculum control or formic inhibition series.

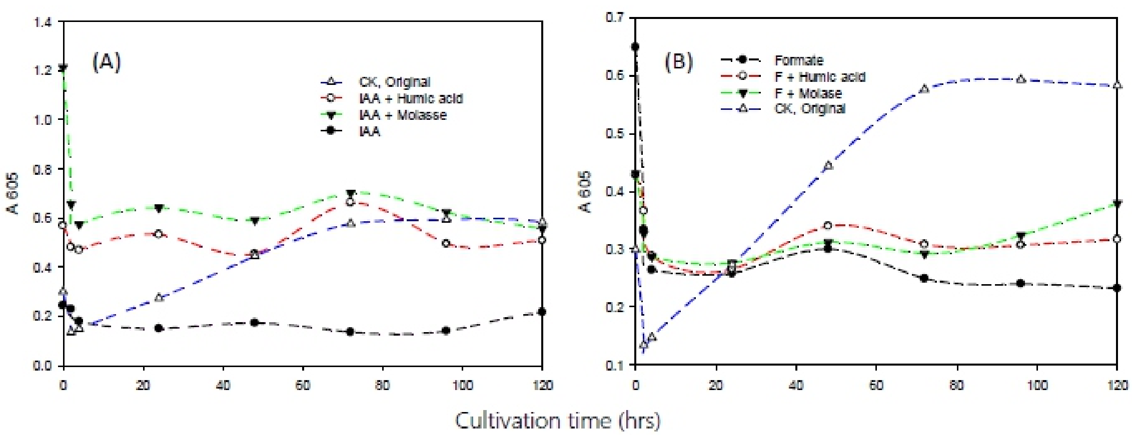

Figure 6. Effects of electron donors and grafting mediators on the growth of active TEX remediation of Bacillus cereus 3-1. A) 20 mM IAA, SHH (A440=0.20), molasses (A440=0.25). B) 20 mM formic acid.

These results indicated that MA with SHH could provide ring and polar graft chains and participate in the remediation process by donating electrons and interacting with oxygen-containing groups. Then, targeting of ring transformation proceeded. This mode of action triggers a shift in electron flux and triggers a low oxygen state, and the growth curve is catabolized via functional groups and slow in cleavage of the ring structure, which is postulated to be secondary metabolic activity [46–48]. Integration through both chemical and biological remediation would be a beneficial way for stable remediation for dealing with soluble hazardous pollutants due to their rapid spread in media penetration.

Since Bacillus spp. were spore formers, bioantagonistic agents for antibiotics, oxygen scavengers, and benefits for cleanup pollutants disseminated in the environment, seriously mounting as petrochemical emission problems. The present study strongly emphasizes the possibility of metabolic products by producing secondary metabolite chemicals; thus, combined formulations were possible for establishing biostatic effects for the soil regime, which are important for the photosphere. The importance of incorporating SHH +IAA by grafting for BM cleanup met the possibility of hydrolytic nanohumus matter in fermentation. These results lead to good proximity for reactants and responses to plant hormones in both plant and microbial routes [3,48]. Molasses, which pertains to a disaccharide-rich source, exerts higher primary metabolism and cannot proceed efficiently in remediation [49]. Our findings indicated that molecular interactions in solution existed for nanosoluble molecules such as SHH with diphenyl polar indole or IAA and stimulated aromatic conversion reactions. These results agreed with reports pointing out that chemicals induce defensive ability under physiological changes without proliferative growth, which supports the driving of cell metabolic activity by regulators and inhibitors [50–52]. In conclusion, a lowered remediation effect with the mediator before the mid-log phase was shown if primary metabolism was elicited. Then, more active bioremediation would be exerted by switching, and changing could be regulated, even after one to three days of latency. Future studies could devote more effort to developing technology for increasing active cells for synchronous growth and increasing cell density during remedial fermentation. Additionally, phytoremediation cases could be surveyed by using this model.

4. CONCLUSIONS

A strain of B. cereus 3-1 with high proteinase activity was purely isolated from a 10-L biofilm reactor under facultative anaerobic conditions for bioremediation of BTEX. The trial of stabilizing remediation with TEX proceeded with SHH, molasses as primary EMD, indole, IAA as secondary EMD and in combination synergistically in fermentation.

Among the revealed reactions, SHH combined with IAA could raise higher activity than MA alone. Bioremedial activity was maintained for 126 hours (5 days) and monitored with 100 rpm rotary shaking and anaerobic fermentation. The kinetic simulation curve supported that the combined melanoid carbon source with electron mediators partially reduced growth, which caused delayed bioremedial activity. The enhancement of remediation observed by combining SHH with IAA, which postulated the effectiveness of electron donation from alkyl grafting, might be interpreted as an increase in the mediation of transformation. MA was utilized in microbial growth at the latter phase but lowered remedial activity was not stably shown even when combined with IAA. Either IAA or formic acid alone alleviated some extent of inhibition, but the combination with SHH raised the activity analyzed even after 76 hours of cultivation, indicating heterogeneous interaction even by combined dosing and mixing, resulting in nonsynchronous growth during the progress of anaerobic cultivation.

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Conflict of interest

The authors declare no competing financial interests.

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