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RESEARCH ARTICLE

Microbial indicators in municipal solid waste compost and their fate after land application of compost

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Abstract

Despite the extensive agricultural use of municipal solid waste (MSW) compost, there is little information about microbial characteristics of the MSW compost and fate of microorganisms following land application. This study was designed to determine the microbial quality and germination index (GI) of the MSW compost as well as fate of indicator microorganisms after application of MSW compost. The results showed a high fraction of samples are immature (GI < 80). In 27% and 16% of samples fecal coliforms and *Salmonella* were detected in a range exceeding the recommended value for unrestricted application of compost, respectively. HAdV was also detected in 62% of samples. Fecal enterococci were detected with relatively high concentrations in all samples and showed higher survival rate than other indicators in land-applied MSW compost. The results showed that climate condition significantly contributed to the decline of indicator bacteria in land-applied compost. The results highlight the need for further quality monitoring of compost to ensure that its application does not lead to environmental or human health problems. Furthermore, because of the high concentrations and high survival rate of enterococci in compost samples, they can be specifically proposed as an indicator microorganism for MSW compost quality monitoring.

Keywords Compost · Pathogens · Indicator bacteria · Survival · Land application · Municipal solid waste

Introduction

Urban development has been led to produce large amounts of municipal solid waste (MSW) that must be managed in suitable conditions to prevent adverse environmental and human health effects [1]. Landfilling, incineration, pyrolysis, biogas and composting are some of the methods for managing and disposal of solid wastes [2].

Among the various MSW management options, much attention has recently been paid to composting process as a valuable method due to the economic and environmental benefits especially in developing countries with a high fraction of waste organic matters [3]. If composting process is properly controlled leads to the production of a stable end product, which can be used as fertilizer for agricultural purposes. In composting process, through biological activity, organic wastes are converted to a humus like matter which could improve physical and chemical quality of soil and recover degraded soils especially in arid regions [1, 3]. However, usefulness of MSW compost application in agricultural fields is depending on whether quality of the product can meet the standards. In other words, MSW compost should be stable and mature in order to prevent some problems like damage to plants due to the presence of phytotoxic compounds, production of unpleasant odors because of the presence of unstable organic matter and the potential for presence or regrowth of pathogens [4].

Ultimate characteristics and quality of the MSW compost are dependent on some factors including characteristics and proportions of the raw materials, the aeration rate, moisture content as well as storage conditions of the composted material. Since the preparation of MSW compost is an important factor for sustainable agricultural and resource management, composting process has to be conducted appropriately in

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order to prevent the adverse effects may induce through application of MSW compost without a good stabilization and maturation process. However, management of composting process and evaluation of the MSW compost quality are grave problems in most of the large cities especially in developing countries because of the generation of vast amounts of MSW. According to the last report of the World Bank, average generation rate of MSW per capita in urban areas is approximately 1.2 kg/person/day and will likely increase to 1.42 kg/person/day in 2025 [2]. There is a little evidence about the quality monitoring and verification of MSW compost in Iran. Furthermore, unpleasant odors of applied MSW compost in urban green spaces may indicate application of immature and poorly stabilized compost which could generate problems of hygiene and phytotoxicity. Immature compost could become anaerobic which leads to odors and/or the development of toxic compounds [5]. Therefore, there is a concern regarding the safety of application of immature and unstable compost and consequently potential contamination of soil and the food chain. On the other hand, some microorganisms such as *Salmonella* and *E. coli* as well as pathogens may regrow in the final compost in storage phase or after spreading on the soil when the thermophilic phase is inefficient and the microorganisms expose to sub-lethal heat shock [6]. In this regard, a complete quality monitoring of composted materials is required to ensure that application of MSW compost does not lead to environmental or human health problems via pathogens, heavy metals, and phytotoxicity.

Despite of extensive agricultural usage of MSW compost worldwide, information on the microbial characteristics of the MSW compost and fate of microorganism after application is rarely available. Therefore, the present study was carried out to 1) determine the microbial quality of MSW compost and germination index as a parameter for phytotoxicity, 2) evaluate the decay rate of MSW compost indicator microorganisms as reference to pathogens presence in the field of a semi-arid region after land application.

Materials & methods

Sample collection

This study was conducted to investigate the physicochemical and microbial characteristics of MSW compost and fate of microorganisms in compost-applied field in Isfahan, Iran. Isfahan is located in the central part of Iran with a semi-arid climate with an average annual temperature of 16.7 °C and an average rainfall of 130 mm/year [7]. A total of 37 samples were analyzed for characteristics of MSW compost. The samples obtained during the period of MSW compost application (December 2019 to March 2020) in public green spaces. Each sample consisted of five subsamples taken from different parts in the pile and were mixed to get a representative sample. All samples were collected in sterile bags, transferred to the laboratory and analyzed for the physicochemical and microbial parameters.

To evaluate the survival time of microorganisms in compost-applied soil, MSW compost (final product) was spread over the lawn in three 4.5×4.5 m plots similar to what is done in public green spaces for four runs in autumn, winter and spring in order to take into account weather (temperature, humidity and UV index) variations on the survival of indicator bacteria. Samples were collected from each plot by randomly selecting several sampling points after 0, 5, 10, 20, 30, and 45 days after spreading of compost. Samples from each plot were homogenized, detritus were removed and analyzed for the presence of indicator and pathogenic microorganisms. In order to investigate the effect of climatic parameters on the rate of microorganism's die-off, information about humidity, ambient temperature and UV index during the experimental period was recorded.

Physicochemical parameters

The moisture content of the compost samples was determined using the amount of weight loss after drying the samples for 24 h at 70 ± 5°C. pH was determined in 1:5 (w/v) aqueous extracts [8].

Phytotoxicity test

Germination index of MSW compost samples as a parameter of maturity and consequently phytotoxicity was determined using watery extracts of compost which are placed on a filter-paper in a Petri disk by cress seeds [9]

Microbial parameters

The most common criteria for microbial quality of compost are fecal indicator bacteria (FIB) and *Salmonella* with reference to pathogen presence [10]. In this study, MSW compost samples were analyzed for microbial parameters including fecal coliforms, *E. coli*, fecal enterococci, *Clostridium perfringens* as indicator microorganisms and *Salmonella*, *Shigella* and human adenovirus (HAdV) as pathogenic microorganisms. For bacterial analyses, 10^{-1} (20 g compost in 180 ml peptone water) homogenized sample of MSW compost was prepared in peptone water. Suspension of compost samples was shaken about 2 h in a shaker incubator at 37 °C, and after 10 min of settling, the suspension was diluted and tenfold dilution series was used for microbial analyses. The number of indicator and pathogenic microorganisms was determined by multiple-tube fermentation technique (MTF) [8, 10]. For

Salmonella, *Shigella* and *Clostridium perfringens* following incubation on the selective agar media, suspected colonies were analyzed by using specific primers in a polymerase chain reaction (PCR) method. Standard isolates of bacteria were used as positive controls [7, 11]. For positive samples, results were reported based on the most probable number (MPN) or colony forming unit (CFU) of detected microorganisms per gram dry weight (gDW) of sample.

HAdV was recovered from MSW compost by polyethylene glycol 8000 following ISO/TS 15,216–2:2019 (E) procedure as described previously [12]. The pellet of viral particles resuspended in 500 µL of PBS and subjected to DNA extraction. Viral nucleic acid was extracted and purified using the High Pure Viral Nucleic Acid kit (Roche, Molecular Biochemicals Ltd, Mannheim, Germany) as per manufacturer's instructions. HAdV was finally quantified by real-time PCR. Real-time PCR analyses were run in duplicates containing negative and positive control [12]. The primers used in this study are listed in Table 1.

Die-off rate of indicator bacteria

Decay rate (die-off) of indicator bacteria including fecal coliforms, *E. coli* and fecal enterococci in MSW compost-applied soil was estimated by the following equation [13]:

$$C_t = C_0 10^{(-kt)} \quad (1)$$

where C_t is the population of microorganisms at time t (5, 10, 20, 30 and 45 days after spreading), C_0 is the initial population of microorganisms, k is the decay rate coefficient, and t is the interval time between the sampling times. Principal component analysis (PCA (performed by R-Studio software to determine the relationship between k (decay coefficient) and environmental parameters.

Results and discussion

Characteristics of MSW compost

For unrestricted application of the MSW compost, certain decisive criteria must be met in composted material in order to minimize risks from handling and application of the products. The physicochemical and microbial parameters of MSW compost are presented in Table 2. In the present study, the lowest and highest moisture content of compost samples was 8.2% and 24.6%, respectively. One of the important parameters for the evaluation of maturity of compost is germination index. Immature compost may contain methane, ammonia or acetic acid, which are harmful to plant growth [5]. GI more than 80% indicates phytotoxic-free and mature compost [14], and if the value is below 50%, the compost

Table 1 Primers used in the study

Target	Primer	Sequence	Size (bp)
C. perfringes	16sF 16sR	GGGGGTTTCAACACCTCC GCAAGGGATGTCAAGT	170
Shigella	*ipaH*-L1 *ipaH*-U1	CCT TTT CCG CGT TCC TTG A CGG AAT CCG GAG GTA TTG C	426
Salmonella	*invA*139 *invA*141	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284
Human adenovirus	AD40/41F HEX245R	CAGCCTGGGGAACAAGTTCA ACTTTGTAAGARTARGCGGTKTC'	129

Table 2 Physicochemical & microbial characteristics of the MSW compost

Parameter (Unit)	Mean ± standard deviation	Min	Max
pH	–	7	9
Moisture content (%)	15 ± 8	1	26
GI* (%)	34 ± 25	0	100
Fecal coliforms (MPN/gDW)	4028 ± 13,373	< 3	58,524
Escherichia coli (MPN/gDW)	4 ± 5	< 0.3	31
Clostridium perfringens (MPN/gDW)	4 ± 10	< 0.3	61
Fecal enterococci (MPN/gDW)	28,026 ± 58,613	5	297,030
Salmonella (MPN/4gDW)	1 ± 2	< 0.3	8
Shigella (MPN/gDW)	ND	ND	ND
Human adenovirus (GC/gDW)	92 ± 232	ND	1298

*ND** Not Detected, *GI** Germination Index, *GC** Genomic Copy

presents a high level of phytotoxicity [2, 15]. The results of study showed a high fraction of the MSW compost are immature (GI < 80) and 73% of samples may induce phytotoxicity (GI < 50). Immature composts have been shown to cause phytotoxicity, possibly due to intermediate organic compounds produced during the initial stage of decomposition. It has been proposed that failure of composting process to sufficiently increase the temperature to the target thermophilic level, may cause the production of immature compost which contains human and animal pathogens as well as weed seeds [16].

As shown in Fig. 1 in some samples, microbial indicators have detected in a range exceeding the recommended value for unrestricted application of MSW compost. 27% and 16% of the MSW compost samples have fecal coliforms and *Salmonella* concentrations higher than 1000 MPN/gDW and 1 MPN/4gDW, respectively. Although a high fraction of compost samples contained *E. coli* (57%), its concentration not exceeded the 1000 MPN/gDW (Fig. 1). Absence of *Salmonella* as a human frank pathogen, is very important in evaluation of the efficiency of composting process and sanitary quality of compost and consequently potential health risks. The presence of this pathogen in finished products leads to the assumption of shortages in the composting process. However, because of low persistence of *Shigella* [17], this bacterium was not detected in the compost samples (Table 2). Absence of *Shigella* is also mentioned in the study of Hassen et al. [18] which is probably due to their rapid disappearance and inactivation during the composting process of municipal solid waste or their absence since the establishment of composting piles.

In contrast, fecal enterococci were detected with a relatively high concentrations with a mean value of 64,039 MPN/gDW in all MSW compost samples. HAdV was also detected in 62% of compost samples (Table 2). It is important to note that real-time PCR detection of HAdV is not representative of infectious viral particles and with assumption a fraction of 1000:1 genomic copy to PFU [19], only 8% of samples contain HAdV number higher than 3 PFU/4gDW (Fig. 1). However, this fraction is related to wastewater and more research is needed about the survival of viruses in composting process and composted materials.

Microorganism's reduction following compost application in green spaces

Figure 2a–d shows the changes in the concentration of indicator bacteria (fecal coliforms, *E. coli* and fecal enterococci) during the four stages of MSW compost spreading on the lawn. According to the Fig. 2a–d, the number of fecal coliforms decreased significantly after 20th day of spreading and reached to very low levels on the 30th day. However, regrowth of fecal coliforms was clearly observed in all stages between days 5 to 20 following spreading of the MSW compost. *E. coli* was observed in very low concentrations in the two stages (Fig. 2a and b) of compost samples applied on land in autumn and regrowth of it was observed on the 5th and 10th day after spreading. *Salmonella*, as a pathogenic microorganism detected only in the MSW composts of second and third spreading stages (Data not shown) and showed a regrowth phase following spreading which reached to an undetectable level on the 20th day. It has been reported that fecal coliforms and *Salmonella* and some other pathogens have the potential to regrow and recolonize in amended soil with biosolids especially during the rainy season or in wet conditions after irrigation [6]. In other words, manual moisture in the form of irrigation or rainfall may be the main reason for regrowth of microorganisms following compost application on land [20]. In the study of Zaleski et al. [21] regrowth of *Salmonella* in biosolids was attributed to the

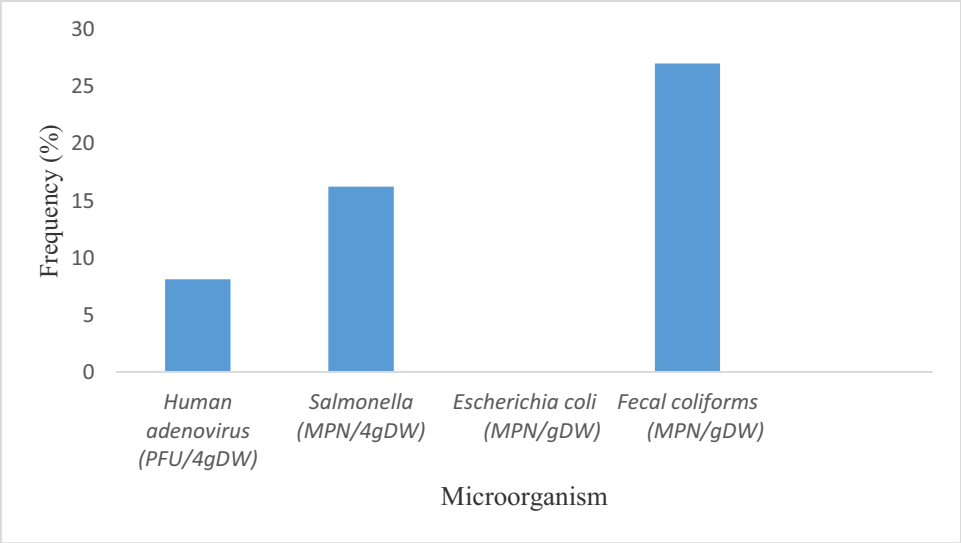

Fig. 1 Detection frequency of microorganisms in compost samples in a range exceeding the recommended value for unrestricted application of compost

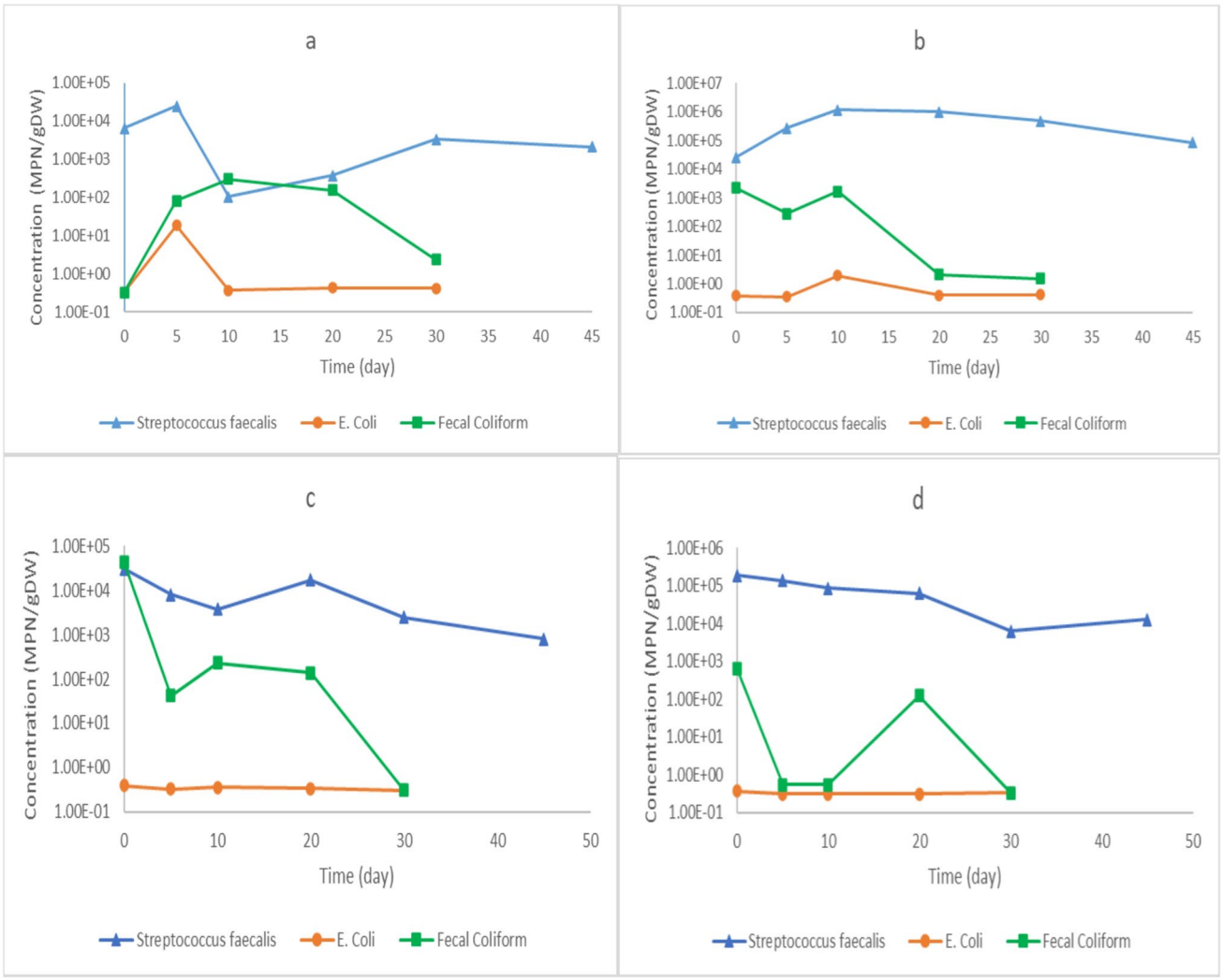

Fig. 2 Trend of change in microorganism's concentration during the MSW compost application on green space (day 0 to day 45 after application), **a** and **b** in autumn, **c** in winter, and **d** in spring

rainfall events. Considering the guideline value of *Salmonella* (3MPN/4gDW) in biosolids class A [17], the concentration of this pathogenic indicator in the MSW compost of second stage (in autumn) and after regrowth was higher than the guideline value. Two or three stages have been reported for die-off of pathogenic or indicator bacteria in soil following application of biosolids, consisting a short period of regrowth for some organisms which followed by a rapid decline and finally a slower decline stage [22]. Protective effect of the soil particles may be the reason for the two-phase decline pattern [22, 23].

Fecal enterococci decreased slower and always remained detectable with relatively high concentrations throughout the monitoring period (Fig. 2a–d). In consistent with our results, it has been found that enterococci survive longer than coliforms [22]. Study of Sossou et al. [24] showed fecal coliform indicators were inactivated more easily during urea treatment than all other indicators and therefore it has been proposed that the use of fecal coliform indicators does not adequately represent the presence of all pathogens. Detection of high concentrations of fecal enterococci in the MSW compost samples as well as long survival time of them in the environment highlight the importance of these bacteria as a criterion for the safety of the compost product. These bacteria were suggested as an indicator organism to assess the efficiency of biosolid disinfection processes [25]. It has been reported that *E. faecium* is a suitable surrogate of *Salmonella* due to its higher thermal resistance [26]. Enterococci with higher resistance to adverse environmental conditions and a limited host range [26, 27] could be good indicators for hygienic quality of biosolids and MSW compost. However, there is controversy about usefulness of these bacteria as an indicator or model microorganism in biosolids and MSW compost [22].

Decay rate of indicator bacteria in the MSW compost-applied soil and effect of environmental conditions

The mean decay coefficients (k) for fecal coliforms and fecal enterococci were obtained as 0.33 and 0.11 per day, respectively, which show the greater resistance of fecal enterococci compared to fecal coliforms. In the study of Farhadkhani et al. [7] the average value of *Escherichia coli* degradation coefficient in a semi-arid region was calculated as 0.27/day which is consistent with the result of present study. Sossou et al. [24] reported the k_{max} at 42 ºC for *Escherichia coli* and enterococci as 1.08 and 0.89 day^{-1}, respectively. In consistent with our results, Sossou et al. [28] reported higher resistance of *streptococcus fecails* than the fecal coliforms.

The fate of microorganisms in application sites and rate of their die-off in amended soil or on crop and soil surface are influenced by a variety of parameters including organism and soil type, indigenous microflora, soil organic matter content and climate conditions including air temperature, moisture, and intensity of UV radiation (UV index) [6, 29] The effect of different environmental parameters including humidity, ambient temperature and UV index on the decay rate of fecal coliforms and fecal enterococci in the MSW compost-applied soil was determined using principal component analysis (PCA) (Fig. 3). However, because of absence or low concentrations of *E. coli* and *Salmonella* in the MSW compost samples applied on soil, decay rate of these bacteria was not estimated. In this study the air temperature and humidity during the experiment were in a range of 8–37 ºC and 8–71%, respectively. UV index varied from 0 to 13 with a mean value of 6. Based on the Fig. 3, the decay coefficients are almost in direction with UV index and ambient temperature and in opposite direction to humidity. In other words, the decay rate of indicator bacteria was increased as the temperature and UV index rose and decreased as the temperature and UV index decreased. PCA model strongly showed that the climatic conditions of arid and semi-arid regions such as high temperature, low humidity and sunlight intensity can effectively inactivate microorganisms on soil surface. In study of Farhadkhani et al. [7] the rate of *E. coli* degradation in soil was affected by ambient temperature and UV index and their correlation analysis showed a direct relationship between the ambient temperature, UV index and *Escherichia coli* degradation coefficient.

Conclusions

The results of study showed regrowth of indicator microorganisms and *Salmonella* after land application of MSW compost. This condition may represent a health concern for the application of MSW compost in urban green spaces. However, climate condition of semi-arid regions including high temperature and UV radiation intensity effectively inactivate microorganisms in the environment. On the other hand, because of the presence of fecal enterococci in high numbers in the compost samples and higher survival rate than fecal coliforms, these bacteria can be specifically considered as MSW compost sanitation indicator.

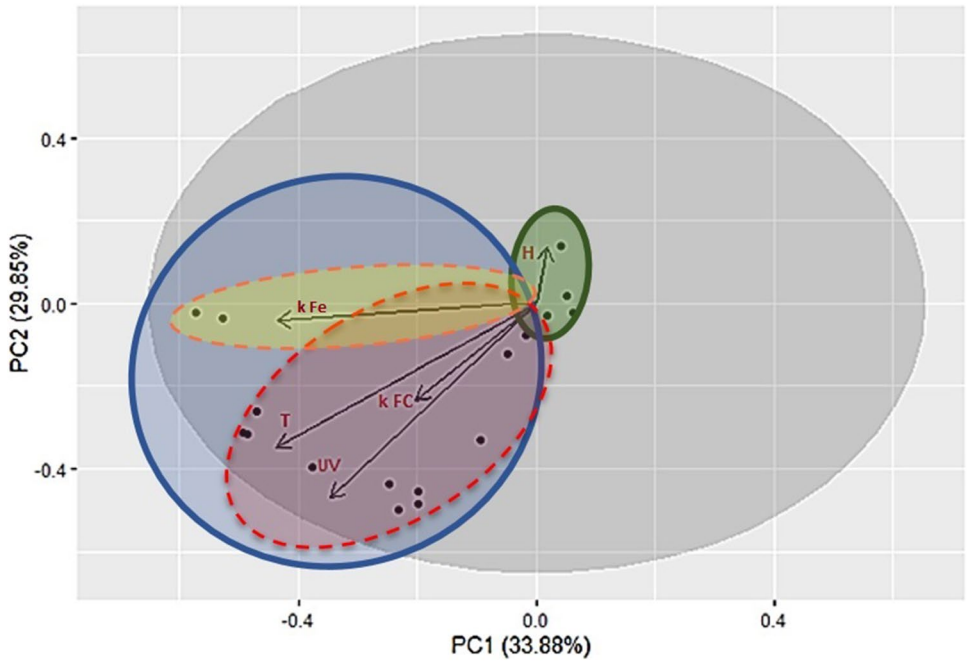

Fig. 3 The effect of environmental parameters (ambient temperature, UV index and air humidity) on bacterial decay rate (fecal coliforms and fecal enterococci) based on the PCA model

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Data availability Data will be made available on reasonable request.

Declarations

Competing interest The authors declare that they have no conflict of interests.

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