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The Role of Biodegrading-bacteria to Remove Bisphenol-A from Polluted Soils

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Bisphenol A (BPA), is an organic component, functions as an endocrine disruptor (EDC). It ubiquitously exists in both, the environment and the food sources, leading to continuous and unintentional exposure among populations of human and animal.
Aim of the Study: Investigate biodegradation activity of BPA by bacteria isolated from polluted soil with plastic wastes.
Materials and Methods: 50 samples were taken from polluted soil with plastic wastes of different sites in Kerbala province; minimal salt media (MSM) were used to identify the capability of the isolates to bioremediate 200 mg/L, as a final concentration of BPA by using HPLC analysis.
Results: the bacterial isolates could degrade the BPA in different ranges started from 18.7% to 99.9%. the recent study found that, *Serratia plymuthica*, *Pantoea spp*, *Shingomonas paucimobilis*, *Pseudomonas aeruginosa* and *Bacillus spp*. were more efficient in BPA biodegradation than *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Escherichia coli* and *Proteus spp*.

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Conclusion: Many types of bacterial isolates could convert the toxic organic compound Bisphenol A to another metabolites and according to recent study we could employee these activity to eliminate these materials safely from the polluted soils and water efficiently than using chemicals that might be toxic to environment, human being and animals.

Keywords: BPA; biodegradation; Sphingomonas paucimobilis; HPLC; minimal salt media; estrogen-mimic.

1. INTRODUCTION

Public Health: Bisphenol A (BPA) considered as an organic substance employed in the manufacturing of resins' epoxy, polycarbonate and also, plastics included polyvinyl chloride; its inception dates back to 1891, gaining widespread utilization about 1930 when researchers developed polycarbonate plastic incorporating BPA successfully [1]. Epoxy resins and polycarbonate plastics found application in various commodities, such as bottles of water, milk and babies, plastic of plates and cups, paints, coatings materials, and packaging for products of dairy; the migration of BPA, inherent in these types of plastics, transpires from the packaging of the materials to the products of dairy over time [2]. Bisphenol A (BPA) did not naturally occur within the environmental milieu; but, its pervasive integration as an industrial constituent in plastic materials had rendered it a ubiquitous element within the global ecosystem; the contamination of the environment by BPA emanates due to various conduits, including effluent wastes from BPA manufacturing processes and the introduction of BPA-laden products into the environmental matrix [3,4]. The disintegration of synthetic polymers, incineration of domestic refuse, deposition of refuse in substantial quantities among other activities, constitute essential sources of BPA influx into aquatic, terrestrial and atmospheric realms; these aforementioned mechanisms collectively gave rise to the propagation of contamination by BPA [5].

Bisphenol A (BPA) serves as an endocrine disturbance (EDC), and ubiquitously present in both the food sources and environmental milieu, leading to continuous and inadvertent exposure for both populations of human and animal to this endocrine-disrupting agent; owing to its considerable impact on health, endeavours had been undertaken to explore substitutes for its utilization [6]. Bisphenol A (BPA) manifest acute toxicity in the animal kingdom; coupled with mutagenic as well as estrogenic implications on human health [7]. Estrogens and their mimics, in addition to inducing heightened expression of the estrogen-receptors, also prompt the upregulation of receptors and related genes and associated with various biochemical entities, including cytokines, peptides and lipids present within the endometrial milieu [8,9,10]. Nevertheless, these deleterious substances were polluting surfaces and subsurface water sources [11].

In vitro investigations and animal trials suggested that, BPA exposure could exert influences on reproductive and cardiovascular systems, besides thyroid functions. additionally, BPA might be associated with metabolic as well as oxidative stress disorders, encompassing diabetes and obesity; furthermore, various detrimental effects of BPA on wildlife had been documented, encompassing developmental inhibition, malformations, and reproductive system dysfunctions [12]. In experimental trials, exposure to BPA had demonstrated detrimental impacts on the reproductive system; specifically, in reproduction of female, neonatal and perinatal; so, exposure to BPA had been documented to induce notable histological modifications in the reproductive tract, disrupt the heat cyclicity, diminish reproductive capacity and induce alterations in hormone levels during adults' life [6,13].

The employment of BPA-degrading microorganisms in bioremediation held promises for the elimination of BPA from contaminated environments [14]. In contrast to conventional chemical remediation methods, bacterial-mediated biodegradation proves cost-effective, environmentally benign and efficacious, even at minimal concentrations [15]. The efficacy of bioremediation hinges predominantly on the selected bacterium's capacity to flourish in the contaminated environments; consequently, the potential of using bacterial strains that, obtained from contaminated soils appears to be promising in addressing the cleanup of harmful and hazardous pollutants [16].

Numerous organisms encompassing bacteria, fungi, algae as well as plants, exhibit the capability to degrade BPA within the environment; nonetheless, bacterial bio-degradation emerges as the most pertinent focus in the realm of biodegradation studies [17]. Within bacterial bioremediation, the efficacy of the process rests upon bacterial metabolism, wherein bacteria derived benefits from pollutant substances as both a source for carbon and substrate for the energy generation by the bacteria; consequently, bacterial biodegradation prove instrumental in the bioremediation process, and facilitating the reduction or removal of environmental concentrations of contaminants [18].

Sphingobacterium sp. and *Pseudomonas aeruginosa* were assessed for their ability to degrade plastics within soil environments; *Sphingobacterium sp.* was known to inhabit diverse habitats such as soil, activated sludge, forests, compost, fecal matter, lakes and various food sources; additionally, these bacterial species had been documented to exhibit potential in the biodegradation of various pollutants, including plastic mixture wastes [19]. Prior researches had demonstrated that, bacteria such as *Sphingobium sp.*, *Pseudomonas putida*, *Bacillus megaterium*, *Arthrobacter sp*., and *Achromobacter xylosoxidans* exhibited the capacity to degrade BPA effectively [20,21].

2. MATERIALS AND METHODS

A) Isolation of Bacterial Strains

1) 50 samples were taken from polluted soil with plastic wastes of different sites in Kerbala province.
2) These samples were prepared and cultured on different media such as (MacConkey agar, blood agar, nutrient agar). For identification of these types of bacteria, the isolates identified by VITEK 2 System.

B) Preparation of Minimal Salts Media (MSM):

Minimal salts media (MSM) was employed to assess the bacterial isolates' capability to degrade BPA, consisting of (1.5 gm) of KH2PO4, (3.5 gm) of K_2HPO_4, (0.5 gm) of (NH4)2SO4, (0.5 gm) of NaCl, (0.5 gm) of $(NH_4)_2SO_4$, and finally (0.15 gm) of $MgSO.7H_2O$. Additionally, trace elements were incorporated, comprising (0.3 gm) $MnSO_4.4H_2O$, (0.2 gm) $ZnSO_4$.7H2O, (0.05 gm) CaCl2.2H2O, (2 gm) of NaHCO3.10 H2O, (0.02 gm) (NH_4) $Mo_7O_2.4H_2O$, (0.5 gm) $CoCl_2.6H_2O$, (0.1 gm) CuSO4.5H2O, and (0.5 gm) $FeSO_4.7H_2O$ dissolved in one litter of distilled water (DW); furthermore, sterilized by using autoclave equipement at 121ºC for 15 minutes in 15 bar. Subsequent cooled to 50ºC, BPA was introduced into the mixture as only carbon source provided at a final Conc. of 200 mg/L. This medium was utilized for the detection of BPA-degrading activity [7].

C) High-performance Liquid Chromatography (HPLC) Analysis

Following the fourth-day incubation period at 37°C, one ml of each bacterial broth culture underwent centrifugation for 10 minutes at 12,000 rpm. The resultant supernatant was then filtered using a 0.22 µm Millipore filter to eliminate insoluble components potentially; then the resulting filtrate was employed for the residual concentration and quantification of BPA [22].

Bisphenol A residual concentration within the culture media was determined using (HPLC) on a column of C18 type, employing (Shimadzu system/Japan). The mobile phase consisted of water and/or acetonitrile, with running time of 25 minutes a totally, and a flow rate set at one ml/min. Elution occurred at a rate of one ml/min, and detection was performed at an absorption wavelength of 220nm. The retention time for BPA was observed between 3.9 to 6 minutes [23].

3. RESULTS

Fifty swabs were taken from polluted soils from different areas in Kerbala province/ Iraq. And these isolates were subjected to examine their ability to degraded bisphenol a by using MSM with BPA as sole carbon source for bacterial growth Table 1.

After inoculation the bacterial isolates to the MSM with bisphenol A. Each isolate that could grow in the Bisphenol A-containing media subjected to identify by VITEK 2 equipment. The results obtained revealed in the following table. Out of 50 samples taken from different sites of polluted soils.

Table 1. Positive bacterial growth grown in Bisphenol-a containing media

No. of isolates	Positive bacterial growth	Negative bacterial growth
50	31 (62%)	19 (38%)

Table 2. The bacterial isolates that grow in Bisphenol a-containing media according to identification by VITEK 2

No.	Bacterial isolates	Number of isolates
1	*Serratia plymuthica*	**5**
2	*Pantoea spp*	**3**
3	*Shingomonas paucimobilis*	**5**
4	*Acinetobacter haemolyticus*	**2**
5	*Acinetobacter lwoffii*	**2**
6	*Pseudomonas aeruginosa*	**4**
7	*Escherichia coli*	**5**
8	*Bacillus spp.*	**1**
9	*Proteus spp.*	**4**
Total number		**31**

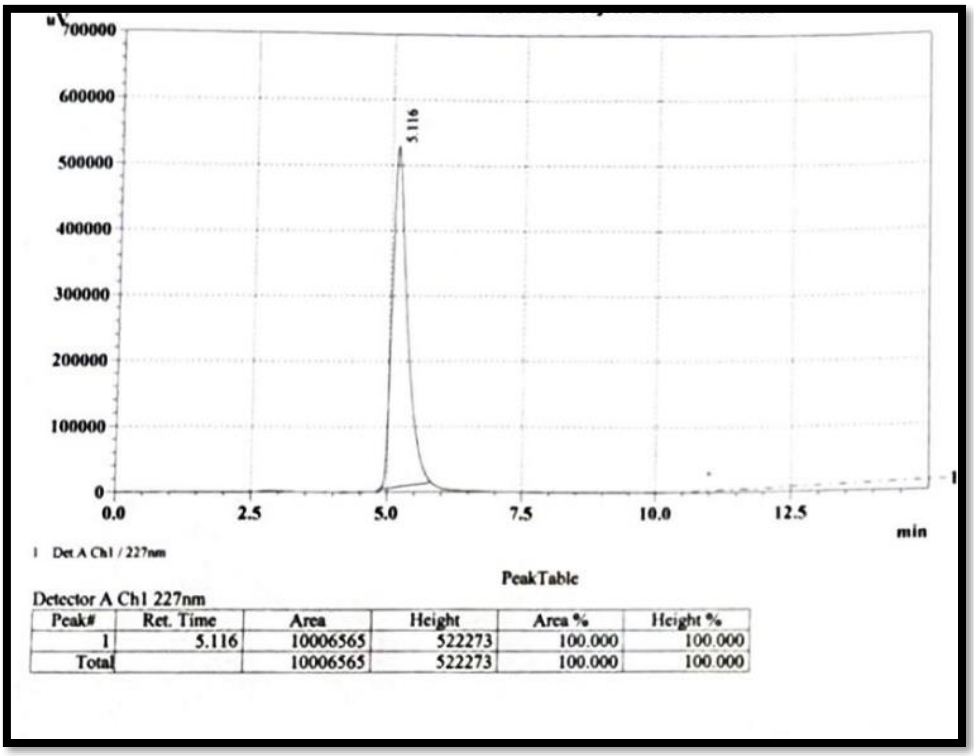
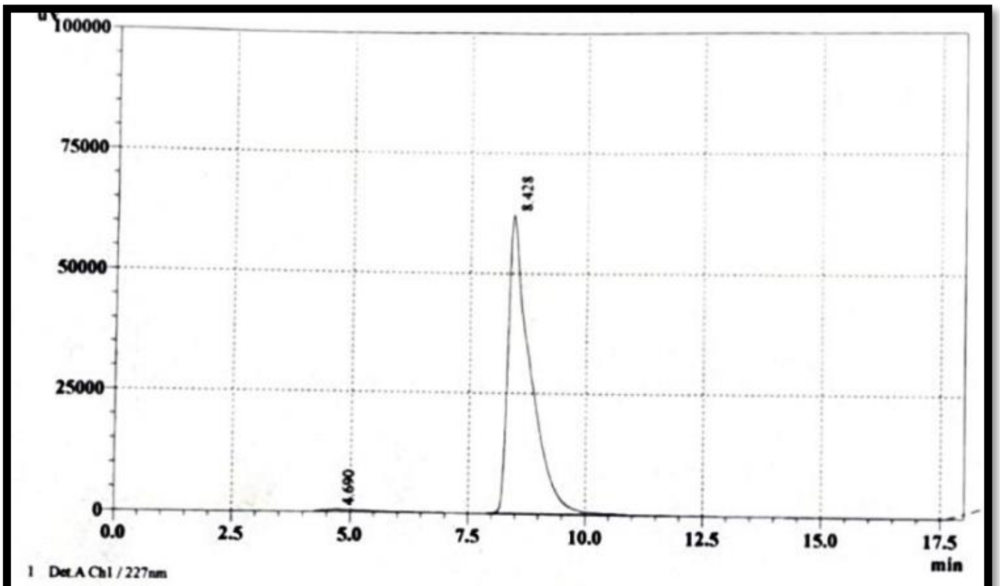


Fig. 1. Bisphenol A control tube, the area under curve and height 100%

After 3-4 days of incubation of bacterial isolates in MSM with bisphenol A. The broth centrifugated to filtrate it from bacterial growth. As well as, Millipore filter used to ultra-filtration. And the residues of BPA in these solutions calculated by HPLC, by using specific column, retention time and mobile phase. Fig. 1, showed the control tube (that was replicated three time for precise results). The tube without bacterial inoculation and determined as a control with area under curve 100% and height 100%; all other tubes that inoculated with bacterial isolates compared to this tube to detect the residues of BPA. Examples of BPA biodegradation showed in Figs. 2 and 3

The results found that, the bacterial isolates could degrade the BPA in different ranges started from 18.7% to 99.9%. the recent study found that, *Serratia plymuthica*, *Pantoea spp*, *Shingomonas paucimobilis*, *Pseudomonas aeruginosa* and *Bacillus* spp. were more efficient in BPA biodegradation than *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Escherichia coli* and *Proteus spp.*

The results of capacity of biodegradation of BPA of all isolates illustrated in the Table 3, as comparing to the control tube.

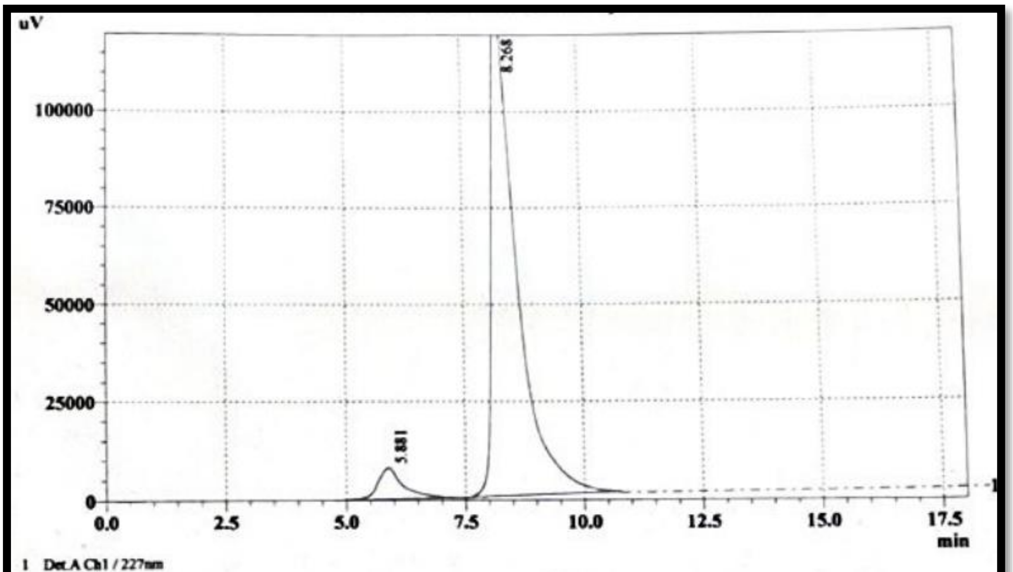


PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.690	2290	55	0.112	0.089
2	8.428	2043508	62132	99.888	99.911
Total		2045799	62187	100.000	100.000

Fig. 2. The ability of *Shingomonas paucimobilis* to biodegrade BPA. using HPLC.



PeakTable

Detector A Ch1 227nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.881	305732	7985	6.178	5.883
2	8.268	4643307	127759	93.822	94.117
Total		4949039	135745	100.000	100.000

Fig. 3. The ability of *Bacillus spp.* to biodegrade BPA using HPLC

Table 3. The Percentage of area, height and biodegradation capacity of bacterial isolates according to HPLC results

No.	Bacteria	Area %	Height %	Biodegradation capacity
1		0.046	0.486	99.9 %
		0.128	0.565	99.8%
	Serratia plymuthica	0.680	1.396	99.3%
		0.821	1.757	99.1%
		74.718	85.274	25.2%
2	*Pantoea spp*	0.410	1.246	99.5%
		2.068	1.457	97.9%
		0.453	0.745	99.5%
3	*Shingomonas paucimobilis*	6.131	5.237	93.8%
		20.026	24.741	79.9%
		0.112	0.089	99.8%
		11.197	9.564	88.8%
		7.681	5.907	92.3%
4	*Acinetobacter haemolyticus*	**67.868**	91.164	32.1%
		65.243	90.341	34.7%
5	*Acinetobacter lwoffii*	**74.160**	93.420	25.8%
		72.166	93.001	27.8%
	Pseudomonas aeruginosa	19.459	28.122	80.5%
		15.182	19.967	84.8%
		17.124	20.235	82.8%
		14.451	19.11	85.5%
6	*Escherichia coli*	**81.262**	89.833	18.7%
		75.458	77.999	24.5%
		80.821	88.122	19.1%
		57.564	85.999	42.4%
		55.754	82.233	44.2%
7	*Bacillus spp.*	6.178	5.883	93.8%
8	*Proteus spp.*	**75.904**	91.071	24.0%
		55.327	88.629	44.6%
		56.21	88.232	43.7%
		66.573	90.122	33.4%
Total Bacterial isolates: 31				

4. DISCUSSION

Bisphenol A (BPA), an organic chemical substances, included with industrial significance, which serving as a crucial raw material in the synthesis of food containers, polycarbonates, industry of thermal paper, epoxy resins, and also, various other products; the extensive utilization of these chemical compounds results in substantial quantities of BPA being released directly into terrestrial and aquatic environments, posing a severe toxicity threat to numerous types of organisms [24].

According to Vijayalakshmi *et al.,* who were observed that, the bacterium *Pseudomonas aeruginosa* demonstrated the ability to thrive in a nutrient broth medium containing varying concentrations of BPA, spanning from 5 mM reaching to 35 mM [25]. The highest BPA elimination rates, amounting of 100% and 96%, were noted under conditions characterized by an initial bacterial amount of 15 ml and a salinity level of 2 percent for concentrations of BPA at 10 and 20 mg/L respectively; the primary metabolites resulting from the biodegradation of BPA, included chloro-benzene, hexa-decanoic acid, chloro-thymol, and 2,6 di-tert-butyl-4,phenylphenol; furthermore, the degradability of the effluent reached 69%, accompanied by a concurrent reduction in toxicity to 7% at all [26].

The BPA-degrading bacterium *Pseudomonas paucimobilis* had demonstrated the capability to mineralize about 60% of the total carbon content of BPA into carbon dioxide (CO2), additionally, it could assimilate 20% of BPA into bacterial cells, while the remaining 20% is transformed into soluble organic compounds [7]. *Enterobacter,* when cultivated in MSM with a singular carbon source of 200 mg/L BPA, exhibited a significant capability for BPA degradation; subsequent to 8, 24, and finally 48 hours of cultivation, the residual quantity of BPA within the cultures was assessed through analysis using HPLC; so, remarkably, these bacteria demonstrated the capacity to biodegrade 53.50±0.153 mg/L of BPA within the 48-hour period, surpassing the degradation observed in the control medium [27].

At least there were four potential pathways for BPA degradation had been identified; the cytochrome P450 monooxygenase system had a notable enzyme complex playing a pivotal role in this process; this system comprises cytochrome P450, ferredoxin and also, ferredoxin reductase; notably, the functions of cytochrome P450 and ferredoxin in this system exhibited similarities to those found in *Sphingomonas* bacteria, as reported by Das *et al.* [28]. According to bacterial biodegradation, the efficacy of the process hinges on bacterial metabolism, wherein bacteria utilized pollutant molecules as solely carbon source and substrate for the energy generation by the bacteria; consequently, bacterial biodegradation emerges as a valuable tool in the bioremediation processes, contributing to the removal or reduction of environmental concentrations of contaminants [29]. Despite advancements in various remediation techniques, it was noteworthy that biodegradation by the bacteria remained the predominant method for addressing BPA contamination; this underscores the significance of harnessing the metabolic capabilities of bacteria in effectively breaking down and mitigating the environmental impact of BPA [30].

The pivotal role of *Pseudomonas aeruginosa* in the biodegradation of various polymers, encompassing degradation of the xenobiotic compounds; moreover, its involvement in the breakdown of plastics, dyes, and oils had been extensively documented; similarly, *Sphingobacterium* had been reported to exhibit notable biodegradation of diverse pollutants potentially; including the ability to contribute to the degradation of mixed plastic wastes; this emphasized the versatility of these bacterial strains in environmental remediation efforts targeting a range of pollutants and substances of polymer-based [31]. In the pursuit of degradation of BPA, beyond chemical and physical treatment methods, microbial degradation had emerged as a focal point of scientific attention; in recent, microbial treatment had gained prominence as an environmentally friendly alternative, offering sustainable approaches to the degradation of plastics, including BPA; this shift towards microbial degradation underscores the potential of harnessing microorganisms to address pollution of plastics and highlights the importance of eco-friendly solutions in strategies of environmental remediation [32].

The analytical study conducted by Yue *et al*. revealed that, while *Sphingomonas spp*. exhibited the capability to fully degrade BPA, the process was deemed inefficient as intermediates tended to accumulate; in contrast, *Pseudomonas sp*. demonstrated more rapid utilization of these intermediates, thereby facilitating the overall mineralization of BPA within the microbial community; this observation cleared the intricate dynamics of microbial interactions and the significance of specific bacterial strains in optimizing the efficiency of BPA degradation pathways [33]. Furthermore, *Bacillus spp*. had the capability to utilizing BPA as the only carbon source; isolated from the creek sediment located within recycling electronic-waste sites, these bacteria demonstrated remarkable efficiency by completely removing 100 percent of 5 mg/L BPA under an optimal aerobic conditions; this underscores the efficiency of *Bacillus spp*. in serving as effective agents for BPA biodegradation, especially in environments associated with electronic-waste contamination [34]. On the other hand, Park and Chin reported that, Bacillus sp. abled to grow in a BPA-enriched MSM; remarkably, Bacillus sp. demonstrated the ability to degrade 84.68% of BPA within a 72-hour period, employing an initial BPA concentration at about 25 ppb; this finding highlights the proficiency of these bacteria in efficiently degrading BPA under controlled environmental conditions [35].

The toxicity bioassay indicated that, the degradation of BPA by *Acinetobacter spp.* not only led to elimination of this component, but also correlated with a reduction in its harmful effects; this suggests that, the tested strains of *Acinetobacter spp*. had served as effective tools for BPA removal in the context of wastewater treatment, emphasizing their significance in mitigating the environmental impact of this pollutant [36]. *Acinetobacter sp*. demonstrated the ability to eliminate 20±3% of BPA at an initial level about 100 mg/L; considering the profound influence of pH values on the activity of the numerous enzymes; their impact on the capacity of BPA degradation by the bacteria were systematically evaluated; it was noted that pH variations might impede the remediation process by blocking the most active sites of the degradation enzymes potentially, thereby inhibiting the efficiency of the BPA degradation processes [37]. As a result, a strain exhibiting significant BPA breakdown capability was isolated and identified as *Proteus mirabilis* throughout this study. Subsequently, it was observed that, these bacteria were able to decomposing 1 mg/L and 20 mg/L of BPA by 98.22% and 66.77%, respectively, within a 72-hour period; this highlighted the efficacy of *Proteus mirabilis* in BPA degradation and expressed its potential applicability in environmental remediation processes [38].

The interactions between BPA and microorganisms throughout the biodegradation process are indeed intricate; Microorganisms exhibited a range of metabolic control mechanisms aimed to countering and neutralizing BPA-induced stress; these mechanisms were orchestrated by the microbial community to adapted to and efficiently degraded BPA, thereby mitigating its adverse effects on the environment totally [39,40].

The examination of bacterial degradation behaviour had predominantly focused on the impact of metabolites such as enzymes and polysaccharides; hence, there are inherent limitations in elucidating the breakdown mechanism of BPA solely by using these methods [41,42]. Indeed, molecular analysis held the promise of providing a more profound and comprehensive understanding of cognition; this approach allowed for detailed exploration of various aspects, as identifying the metabolites involved in bacterial degradation functions, elucidating the pathways employed by bacteria for degradation of pollutants and uncovering the modes of bacterial transport to pollutants; by delving into these molecular intricacies, researchers can gain deeper insights into the mechanisms and processes underlying bacterial degradation of pollutants, contributing to more nuanced comprehensions of cognitive functions in this context [43,44]. Hence, there remained a notable gap in research concerning the evaluation of biodegradation mechanisms from a sufficiently comprehensive perspective. [45].

5. CONCLUSION

Many types of bacterial that isolated from polluted soils with plastics could convert the toxic organic compound Bisphenol A to other metabolites and according to recent study we could employee this activity to eliminate these materials safely from the polluted soils and water efficiently than using chemicals that might be toxic to environment, human being and animals.

ETHICAL APPROVAL

The research was carried out according to ethical principles rooted in the Helsinki Declaration. The protocol of this study, along with the subject information underwent scrutiny, and received approval from a local committee ethics; identified by the reference number (UOK. VET. HE. 2023.067).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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