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Biodegradation of Glyphosate by Four Plant Growth Promoting Bacteria (4PGPB)

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ABSTRACT

Glyphosate was a non-specific organophosphate pesticide, which finds widespread application in shielding crops against the weeds. The degradation of glyphosate in soil usually carried out by microbial activity. Soil Pesticide bioremediation is the most economical, environmentally friendly and successful method available. In this research, four types of bacteria as Bacillus megaterium, Bacillus subtilis, Rhizobium sp., and Azotobacter sp.. The four plant growth promoting Bacteria (4PGPB) were found abundantly in soil. To study the decomposition of the glyphosate pesticide under laboratory conditions and at different concentrations starting from 5ppm, 10ppm, 15ppm, to 20ppm. These bacteria were grown on mineral salt media in 60 days incubation for the experimental condition in the presence of different concentrations of glyphosate. The removal efficiency of the Glyphosate depending on the starting concentration were obtained about (0, 60, 80.5 and 99.98%) for 5ppm, (0, 60.98, 79.80, and 96.80%) for 10 ppm, (0, 51.80, 71.80, and 88.95%) for 15 ppm , and (0, 47.94, 63.94, 87.28%), for 20 ppm respectively, via using the Bacillus megaterium bacterial, while the removal efficiency with values of (0, 59.70, 83.99 and 99.00%) for 5 ppm, (0, 49.87, 82.87, and 93.19%) for 10 ppm, (0, 52.45, 77.45, and 84.99%) 15ppm, and (0, 51.48, 71.48, 75.12%) for 20 ppm, respectively, for the Bacillus subtilisat bacterial. Besides, the removal efficiency for the Glyphosate with values of (0, 96, 86 and 92%) for 5ppm, (0, 57, 80, and 86 %) for 10ppm , (0, 47, 74, and 85 %) for 15ppm , (0, 47, 72, and 84 %), and (0, 45, 67, 80 %) for 20 ppm via using the Azotobacter sp.bactira, respectively. The biodegradation using Rhizobium sp. at 60 day incubation with the same experimental conditions which was (0, 96, 86 and 92%) for 5 ppm , (0, 57, 80, and 86%) 10 ppm, (0, 47, 74, and 85%), (0, 47, 72, and 84%) for 15 ppm , and (0, 45, 67, 80%) for 20ppm from the Glyphosate concentration. The removal efficiency increased with increasing incubation time and with the decreasing of Glyphosate concentrations when used it as a source of phosphorus and carbon for the three plant growth promoting Bacteria (4PGPB). The biodegradation of Glyphosate in soil may be effectively utilized for bioremediation or biodegradation for duration of 30 to 60 days.

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INTRODUCTION

Saving fresh soil is a big challenge for the next generation due to enhanced living standards and population growth. In addition, the expansion of agricultural and industrial activities is causing unmatched demands for fresh water supplies across Iraq. Through industrial processes, a large number of synthetic organic compounds have been discharged into the environment. The most significant environmental agencies focus on environmental monitoring for several classes of organic pollutants, including organic dyes, pharmaceuticals, polycyclic aromatic hydrocarbons, polychlorinated pesticides, polychlorinated dibenzodioxins, dibenzofurans, and biphenyls, due

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to the serious risks they pose to the health of humans and the environment (Abdul Hassan *et al.,* 2019). The pesticides risks are environmental pollution due the leaching in to soils water bodies. Some studies showed low leaching risk glyphosate due strong sorption characteristics, while other studies indicated a leaching risk into deeper soil layers that could end up in surface water and groundwater (Mousa *et al.,* 2019).

Of the various treatment methods available for getting rid of organic pollutants, bioremediation is one of the most promising ways to get rid of these pollutants using natural living organisms such as bacteria, fungi, and plants. Bacteria play an important role in removing organic pollutants and converting them into natural biological materials that can be used later by living organisms(McGuire, et al, 2016).

Organophosphate pesticides are heterogeneous compounds, containing a phosphoric acid derivative. Glyphosate is one of an organophosphate and non-selective herbicide, applied to the leaves of plants for killing both broadleaf plants and grasses (Newman *et al.,* 2016). Several annual and perennial weeds can be controlled using glyphosate ($C_3H_8NO_5P$), a postemergence broad-spectrum herbicide that is nonselective. Because of its minimal toxicity to creatures other than the intended target, it is one of the most widely used herbicides worldwide in urban, forestry, and agricultural settings. In the year of 2014 saw 126 million kg of glyphosate used overall for both agricultural and non-agricultural purposes (Feng *et al.,* 2020). Some detrimental consequences on plants, animals, and human health have been documented as a result of the extensive usage and environmental buildup of glyphosate. These impacts include the weakening of plant systems, disturbance of terrestrial and aquatic animal metabolism, and endocrine disruption in humans (Tarazona *et al.,* 2017; Van Bruggen *et al.,* 2018). Its low molecular weight, low volatility, heat liability, and strong water solubility make it a challenging herbicide to detect in trace analysis. These characteristics make it difficult to extract, purify, and determine (Kaczyński and Łozowicka, 2015).

One treatment option that shows promise is the use of microorganisms to eliminate toxins from polluted areas. Bioremediation is a recognized alternative technique that is favored because to its efficaciousness, minimal harmful, economic worth, and environmental safety (Valavanidis, 2018). Numerous researchers have discovered that certain bacterial groups have a strong capacity to degrade organophosphorus pesticides and other substances (Melo *et al.,* 2018). Therefore, the study objective were to determine and examine the effectiveness of different concentration of glyphosate by using four Plant Growth Promoting Bacteria (4PGPB) in soil and to find any residues using high performance liquid chromatography technique (HPLC) in a lab setting across and at a range of time periods.

MATERIAL AND METHODS

Chemicals and Instrumentations

The important chemicals and reagents were provided from the ministry of science and technology in Iraq laboratories. Glyphosate supply from Iraqi market and the bacteria was grown on mineral salt media (MSM) as shown in Table (1) with glyphosate at concentrations (5, 10, 15, 20) ppm, and the bacteria was grown on mineral salt media (MSM) without glyphosate (phosphorus source) examining their growth and degradation (Sperber**,**1957). The glyphosate degradation by 3PGPB detected its residues by using HPLC analysis.

The 4PGPB Species, Growth, and Biodegradations

Plant Growth Promoting Bacteria (PGPB); *Bacillus megaterium* (Nieminen *et al.,* 2007; Sun *et al.,* 2010*), Bacillus subtilis* (Gachande and Khansole, 2011), *Rhizobium sp.* (Aquilantia *et al.,* 2002; Panwar *et al.*, 2012) and *Azotobacter sp.* (Singh, 2011; Tang and You, 2012) were kept and incubation in laboratories of Remediation Pollutants Center, with known of them by morphological and biological tests, re-cultured before used on MSM. By using the

Table 1. The Mineral Salt Media.

Compounds	Weight/gm
KH_2PO_4	0.2
K_2HPO_4	0.5
$(NH_4)_2SO_4$	1
$MgSO_4•7H_2O$	0.2
NaCl	0.2
$CaCl_2•2H_2O$	0.05
$FeSO_4•7H_2O$	0.025
Na_2MoO_4	0.005
$MnSO_4$	0.005

Table 2. The HPLC Analysis Conditions.

Manual Injector Equipped	20-µL
Column Stationary phase	(C18,ZORBAX),(5µm; 150 mm×4.6 mmi.d.)
Mobile Phase	Acetic acid (1%) &Methanol (60:40v/v).
Flow Rate	1.0 ml /min
Temperature	25°C

spectrophotometer at wave length of 600 nm for measured the bacteria ability growth on MSM with different concentration of Glyphosate (0, 5, 10 and 20 ppm) and without it (Islas *et al.,* 2014). The Glyphosate biodegradations ratio (Islas *et al.,* 2014) was measured by spectrophotometer at wavenumber of depending on the bacteria growth to measured.

HPLC Analysis

The concentrations of Glyphosate in aqueous solution was analyzed by high pressure liquid chromatography technique (HPLC).The Table (2) presented the experimental condition. The samples were withdrawn at the reaction times and analyzed by HPLC at 25 °C. The Glyphosate extracted from the MSM samples were withdrawn after 60 days via using the ethanol as solvent. Added 3ml from the Glyphosate extracted to the conical flask, then added 3ml from the ethyl acetate (EA- extraction reagent), shaken twice time replied. The mixture of suspensions was centrifuged at 3000 rpm /10 min, then filtrated through Whatman GF/B filter (Sperber, 1957). Each of EA extraction was analyzed by HPLC (Shweta *et al.,* 2017).

By using equation (1), the removal efficiency (R%) for Glyphosate using different microbial bacteria was determined. When: $\mathbf{C_0}$ = the initial concentration of Glyphosate in sample (ppm), C_t= the concentration of Glyphosate after time from biodegradation, with concentration of (ppm) then analyzed via the UV-Vis Detector at 254 nm.

$$\text{The Removal Efficiency (R) \%} = [C_0 - C_t/C_0] \times 100\ \% \quad (1)$$

RESULTS AND DISCUSSION

Preparations series concentrations of Glyphosate (5, 10, 15, 20 and 25) ppm to have done the standard curve calibration. As shown in Figure (1), the calibration curve was drawn using various Glyphosate concentrations.

The chemical formula of Glyphosate or (N-phosphono-methyl glycine) can be expressed as follows $C_3H_8NO_5P$ or

The effect of Glyphosate concentration was studied under the 60 days incubation at room temperature. The 4PGPB were grown on MSM for 60 days with Glyphosate. Figure (3A)

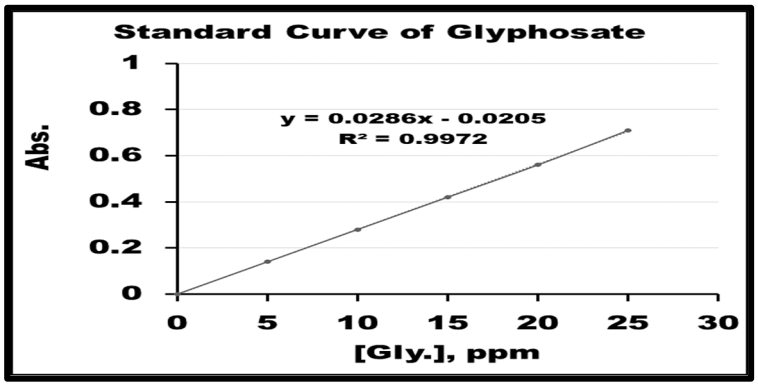

Fig. 1. Standard Curve of Glyphosate.

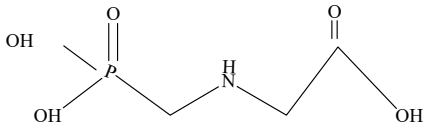

Fig. 2. The chemical formula of Glyphosate

illustrates the effect of Glyphosate concentration (5, 10, 15, and 20 ppm) on removal efficiency via the biodegradations process which was (0, 60, 80.5 and 99.98%),(0, 60.98,79.80,and 96.80%),(0, 51.80, 71.80, and 88.95%), and (0, 47.94, 63.94,87.28%) respectively, at biodegradation using *Bacillus megaterium* bacterial at (0, 15, 30 and 60 day) inception, respectively. From Figure (3B) asshown the effect of Glyphosate concentration(5, 10, 15, 20 and 25mg/L) on the removal efficiency which was (0, 59.70, 83.99 and 99.00%),(0, 49.87, 82.87, and 93.19%),(0, 52.45, 77.45, and 84.99%), and (0, 51.48, 71.48, 75.12%) respectively, by biodegradation using *Bacillus. Subtilis*at (0, 15, 30 and 60 day) incubation, respectively. The removal efficiency which was (0, 69, 76, and 85%),(0, 43,63,and 78%),(0, 44, 63, and 74%), (0, 41, 52,65%), and (0, 34, 50,60%) respectively, at biodegradation using *Rhizobium sp.* at 60 day incubation, respectively, as shown Figure(3C), While the removal efficiency of glyphosate concentration (5, 10, 15and 20 ppm) which was (0, 96, 86 and 92%), (0, 57, 80, and 86%), (0, 47, 74, and 85%), (0, 47, 72, and 84%), and (0, 45, 67, 80%) respectively, as shown in Figure (3D) as shown the effect of by biodegradation using *Azotobacter sp.* at (0, 15, 30 and 60 day) incubation, respectively. From the results obtained we showed that the biodegradation removal efficiency of glyphosate increased with increase of incubation time and with a decrease in glyphosate concentration by using *Bacillus megaterium, Bacillus. subtilis, Rhizobium sp.,* and *Azotobacter sp*., respectively. The removal efficiency of glyphosate biodegradation by using *Bacillus megaterium* is better than that of other bacteria. Figure (4) as shown the HPLC Glyphosate residues for (30-60 days) incubation.

The mainly fact explanation microorganism pesticides degradation is based on enzymes effect on structure of them that led to substrates in soil; depending on microbial population size and not all microbes can metabolism these products tend to persist. suggested used Tetrachlovinphos (TCV)-organophosphorus pesticides as carbon and energy sources (Zhu *et al.,* 2019) .*Flavobacterium sp.* isolated from soil, in OP pesticides contact generated Phosphotriesteras enzyme resulting as metabolic pathways degradation. The co-metabolism microorganism can be the bacteria enzymes converted subtract to organic products that will utilize in energy production, also that substrate transferred to compound inhibit mineralization activity of enzymes (Zhu *et al.,* 2019). *Bacillus megaterium* degradation ability each of

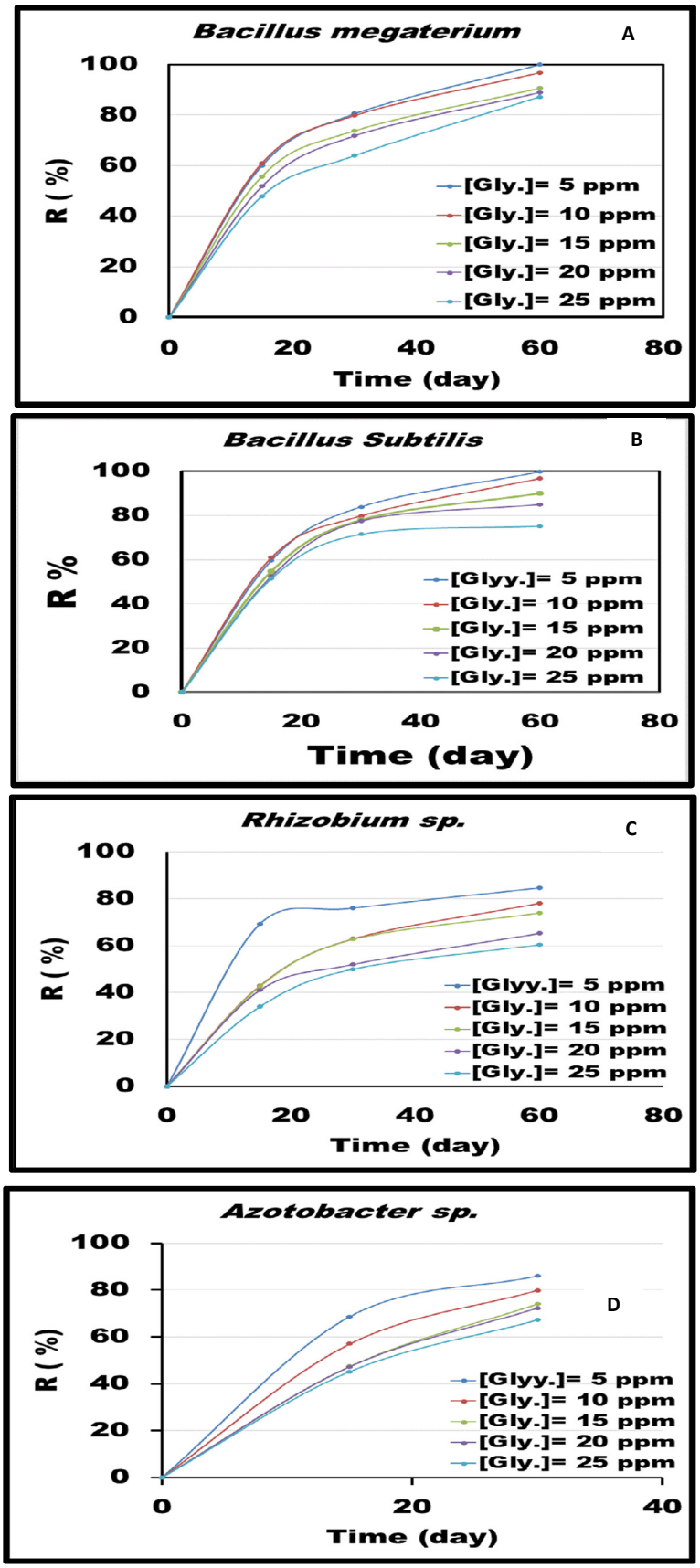

Fig. 3. The Removal Efficiency of Glyphosate Biodegradation using different bacterial.

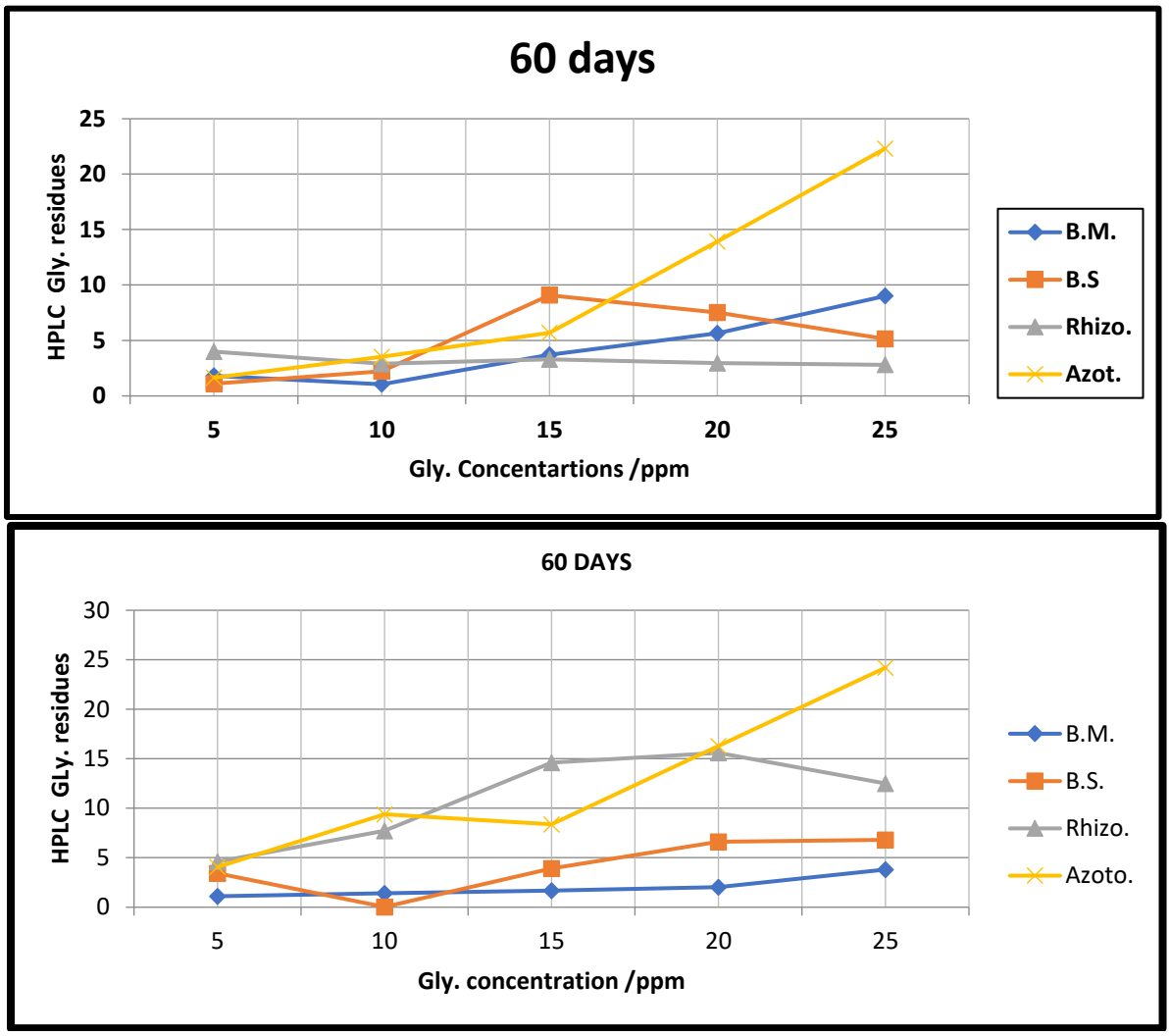

Fig. 4. The HPLC Glyphosate residues for 60 day incubation.

Chlorpyrifos (600ppm/240hrs.); atrazine (50mg/kg for 168 hrs.), and Monocrotophos (MCP) reached (81,99,83) %, respectively, also Chlorpyrifos degradation via *B.megaterium* showed for (7-14 days) (Moneke *et al.,* 2010; Chandrashekar *et al.,* 2017).

Almost, microorganisms increasing growth with increase in glyphosate increasing concentration while reduction growth was with *B. subtilis* (Van Bruggen *et al.,* 2018). *Azotobacter sp.* significantly grew at low glyphosate concentration (7.2-25) x 10^{3} ppm), while at (1-25) x 10^{4} ppm) so inhibition the growth at incubation period. (0-8days) (Chennappa *et al.,* 2014). Fourteen strains of isolated *Azotobacter*, just five strains grew on media containing higher concentrations reach 5% of each glyphosate. phorate, pendimethalin and others isolated from soils (pH6.5-9.5). The results of the present study suggest that the PGPB are capable growing in medium utilizing pesticide as phosphor and / or carbon sources led using for bioremediation of pesticide contaminated soil.

CONCLUSION

The results of PGPB growth showed at 60 days showed the best of the growth bacteria was the *B. megaterium* in concentration of 0.167 ppm . Besides the second bacteria was the B. *subtilize* at concentration of 0.2 ppm .On the other hand, the Rhizobium *sp*. showed 0.191

ppm, while the *Azotobacter sp*. bacteria showed growth of 0.163 ppm, in the same experimental conditions.

The best glyphosate removal efficiency for PGPB by using *Bacillus.megaterium* at (5-20 ppm/60 day incubation time) reached about (99.98-87.28)% ,while the *removal* efficiency *of* glyphosate via the *Bacillus.subtili at concentration of* (5-20 ppm/60 day incubation time) was (90-75)%. Besides, using of the *Rhizobium sp*. at the same concentration of glyphosate (5-20 ppm / 60 day incubation time) reached about (85-50.60) % respectively, while the removal efficiency of glyphosate reached 92-80)% by using *Azotobacter sp.* The biodegradation removal efficiency of glyphosate increased with increase of incubation time and with a decrease in glyphosate concentration by using *Bacillus megaterium, Bacillus. subtilis, Rhizobium sp.,* and *Azotobacter sp*., respectively. The removal efficiency of glyphosate biodegradation by using *Bacillus megaterium* was better than that of other bacteria. The best residues of glyphosate analysis by PGPB were: *Bacillus megaterium*: then the B. *subtilis* , *Rhizobium sp* and *finaly the Azotobacter sp.* in the same experimental conditions, respectively. The *Rhizobium sp*. at the same concentration of glyphosate (5-20 ppm / 60 day incubation time) reached about (85-50.60) % respectively,

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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