Bioremediation of Polluted Water with Pendimethalin Herbicide

*[1]Ibrahim, Ibrahim S. and [2]Ibrahim M. Gomaa

[1]Plant Protection (Pesticides) Dept. Fac. Agric. Al-Azhar Univ., Cairo, Egypt

[2]Environment and Bio-agriculture Depart. Fac. Agric. Al-Azhar Univ., Cairo, Egypt

*Corresponding Author: i.said@azhar.edu.eg

Abstract: The efficacy of *Bacillus halotolerans* bacterium strain MSR-H4 and *Azolla pinnata* 7001 plant used separately or in combination on the remediation of water polluted with pendimethalin herbicide at 400 mg/l was examined. Four treatments were conducted pendimethalin-polluted water (control), pendimethalin-polluted water with *Bacillus halotolerans* strain MSR-H4, pendimethalin-polluted water with *Azolla pinnata* 7001 and pendimethalin-polluted water with *B. halotolerans* strain MSR-H4 and *A. pinnata* 7001 both. For each treatment, the degradation percentages of the herbicide were determined after 7, 14 and 21 days using GLC. Results showed that at each time, the efficacy of treatments on herbicide degradation could be arranged as follows: combination of *B. halotolerans* strain MSR-H4 and *A. pinnata* 7001> *A. pinnata* 7001> *B. halotolerans* strain MSR-H4. Moreover, the degradation rate was increased with time. The highest degradation percentage of pendimethalin was 96.66% when *Bacillus halotolerans* strain MSR-H4 and *Azolla pinnata* 7001 were used together after 21 days.

Keywords: Bioremediation, Pendimethalin, *Bacillus halotolerans*, *Azolla pinnata*

1. Introduction

Excess usage of chemical pesticides leads to pollute a wide range of water and terrestrial ecosystems. Pendimethalin [N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenamine] is synthetic herbicide used as pre-emergence or early post-emergence for the selective control of most annual grasses and broad-leaved weeds in various crops. Pendimethalin has classified as persistent bio-accumulative toxicant according to the U.S Environmental Protection Agency, **(USEPA, 1999)**. This herbicide, a common contaminant of water and soil, is also toxic to fish and aquatic invertebrates **(Meister, 1992)**.

The half-life of pendimethalin herbicide in soil is about 60 days under tropical field conditions. Its widespread use and persistence lead to accumulate this herbicide in soil and water **(Larson *et al.*, 1999 and Racke, 2000).**

Remediation processing becomes essential when accumulation of toxic substances in water and soil is beyond permissible limits. Microorganisms or plants are generally used in the bioremediation and detoxification of many toxic xenobiotics from polluted sites in the environment **(Jamaluddin *et al.*, 2012)**.

Phytoremediation is relatively new approach that uses plants to remove pollutants from soil and water **(USEPA, 1999 and Raskin and Ensley, 2000)**. This approach is emerging as an innovative remediation of pollutants, because plants are solar-driven and thus make this technology a cost-effective mode and eco-friendly. The floating aquatic plants were the greatest known potential for treatment polluted waters includes water hyacinths (*Eichhormia crassipes*) **(Jamuna and Noorjahan, 2009)**, Water Lettuce (*Pistia stratiotes*), **(Awuah *et al.*, 2004)** and water ferns.

Azolla is a genus of aquatic ferns that native to Asia, Africa and America. *Azolla* species live in lakes, swamps, streams and other water. Under suitable field condition, *Azolla* can double its weight every 3-5 days. The use of aquatic plants, such as *Azolla* with hyper accumulating ability is known to be an environmentally friendly option to remediate polluted water **(Anjuli *et al.*, 2012)**.

Bio- and phyto-remediation have been used by scientists as two independent "green technologies", employing separately either microorganisms (bacteria and/or fungi), or plants to reclaim polluted soil, water and air. The two technologies are working synergistically to obtain better results in terms of reclamation performances. Therefore, the single term bioremediation can be used to refer to both of these technologies.

Many researchers have used microorganisms or plants to remediate pesticides contamination. But few of researchers have used microorganism and plant together to remediate of the pollution. Most previous studies focused on using of *Azolla* in phytoremediation of heavy metal from aquatic ecosystems **(Premla *et al.*, 2018)**.

Therefore, the present investigation was conducted to use *Bacillus halotolerans* bacterium strain MSR-H4 and *Azolla pinnata* 7001 plant either separately or together for bioremediation of pendimethalin-polluted water.

2. Materials and Methods

2.1. Experimental location and source of water

The experiment was carried out in the experimental farm of Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, during 2019. Nile irrigation water of the farm was the source of water.

2.2. Pesticide used

The formula of pendimethalin herbicide (Stomp extra 45.5% CS), N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) was obtained as a gift from BASF company. The structural formula of pendimethalin was illustrated in figure (1).

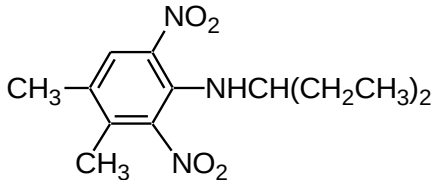

Fig (1): Structural formula of pendimethalin

2.3. Bacterial strain

Bacillus halotolerans strain MSR-H4 a new active bacterial strain plant growth promoting rhizobacteria (PGPRs) was obtained from Department of Agric. Microbiology, Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt. *B. halotolerans* strain MSR-H4 was grown in Luria-Bertani (LB) broth medium then incubated for 48 hr at 28˚C (log phase), cell densities were adjusted to be 30×10^7 colony forming units (cfu)/ml.

2.4. Plant used

Azolla pinnata 7001 was provided by Soil, Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt.

2.5. Experiment

The experiment was carried out in plastic containers (15cm×15cm×10cm, 2000 ml capacity) containing 1000 ml of water treated with pendimethalin at 400 mg/l (this concentration was selected according to previous studies). The treatments were as follows; pendimethalin-polluted water (control), pendimethalin-polluted water with *Bacillus halotolerans* (10 ml/replicate; cell densities were adjusted to be $30x10^7$cfu/ml), pendimethalin-polluted water with *Azolla pinnata* 7001 (10 gm/replicate) and pendimethalin-polluted water together. Each treatment included three replicates. Samples (100 ml) were taken from each treatment after 7, 14 and 21 days of treatment. Samples were extracted at regular intervals for analysis of the pendimethalin residues by GLC. The volume of the pendimethalin-polluted water in each container, for each time interval, was kept constant by adding water compensating for water lost through plant transpiration and evaporation **(Huilong and Xiangjuan, 2006)**.

2.6. Extraction of pendimethalin

A known volume of treated water samples (100 ml), for each time interval, was filtered and transferred into 250 ml separatory funnel. Afterwards, 40 ml of hexane: acetone mixture (3: 1 respectively) were added followed by 10 ml saturated sodium chloride solution (20%) and 1.0 ml methanol. The content was vigorously shaken for five minutes and allowed to stand until separation of layers. The hexane layer was collected in a clean bottle and the aqueous layer was re-extracted twice with 20 ml hexane. Hexane fractions were recombined in a clean bottle and dried up by passing through anhydrous sodium sulphate on a filter paper. The solvent was concentrated to near dryness and the residues were re-constituted in 1ml hexane and stored in the refrigerator at 5°C for chromatographic determination by GC, **Abdullatief *et al.* (2015)**. The extraction efficiency of the methods established, a recovery experiment was conducted in the water spiked with known concentrate of pendimethalin. The recovery percentage of pendimethalin was 90%. The obtained results were corrected according to the recovery percentage.

2.7. Gas liquid chromatographic analysis

Aglient GLC model 7890 was used for determining the residues of pendimethalin. The residues were dissolved in 1 ml of methanol; 1µl of methanol extract was injected into GLC equipped with a Ni^{63} – electron capture detector. GLC condition: column PAS-5 (30 m length x 0.25 mm internal diameter x 0.25 µm film thickness) was used, carrier gas: N_2 at a flow rate of 1.7 ml/min; injector and detector temperatures were 250˚C and 300˚C respectively. The initial column temperature was initial oven temperature: 40˚C for 2 min, raised at 5˚C/min and then held at 260˚C for 5 min. The retention time for pendimethalin under this condition was 9.86 minute as shown in Fig. (2).

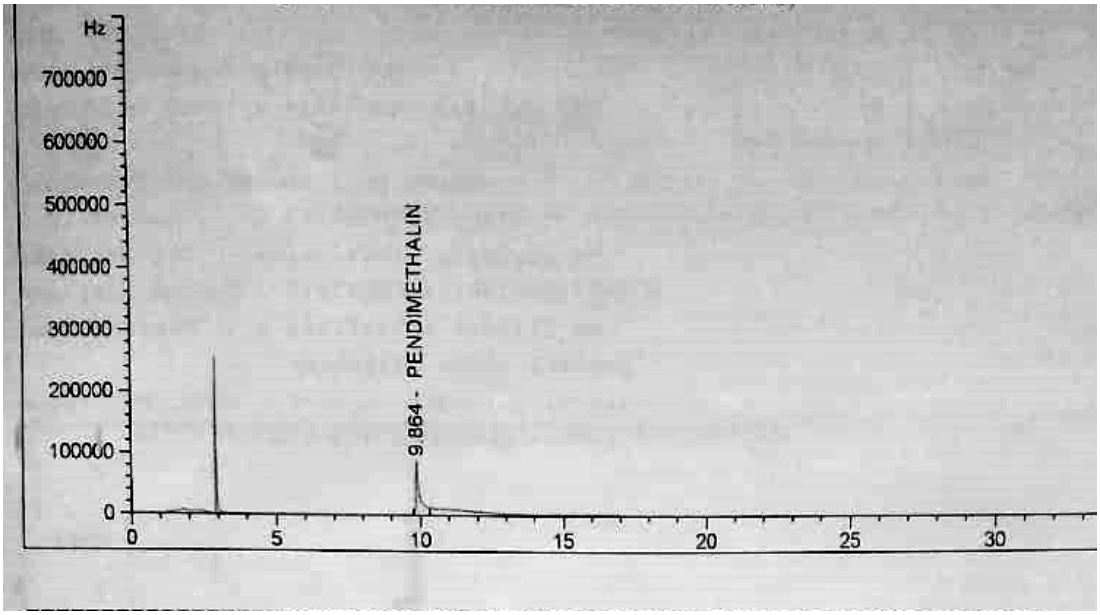

Fig. (2) Standard peak curve of pendimethalin

2.8. Calculation of pesticide biodegradation

The percentage of pesticide degradation was calculated by the following equation:

$$X\% = \frac{C_{ck} - C_x}{C_{ck}} \times 100$$

Where, X is pesticide degradation; C_x is the concentration of pesticide (mg/l) in the treatment; C_{CK} is the concentration of pesticide (mg/l) in the control.

2.9. Statistical analysis

The obtained results were statistically analyzed by two–way of variance (ANOVA) using CoStat statistical, computer program software, to determine least significant difference (L.S.D.) at 0.05 and 0.01 confidence degree.

3. Results and Discussion

The degradation of pendimethalin at 400 mg/l was examined in pentamithalin-polluted water using *Bacillus halotolerans* strain MSR-H4 bacterium and *Azolla pinnata* 7001 plant either separately or together. As shown in Table (1) and figure (3), inoculation of pendimethalin-polluted water with *B. halotolerans* strain MSR-H4 alone resulted in a significant higher pendimethalin degradation rate than that of the uninoculated treatment (control). Furthermore, pendimethalin degradation rate remarkably increased by increasing the time. The obtained results agree with **Chirom *et al.* (2018)** who found that pendimethalin concentration (500 mg/l) declined with increasing incubation time in the medium treated with *Bacillus cereus,* than in the control. Also, Table (1) shows that at each time, *Azolla pinnata* 7001 gave more pendimethalin degradation than that of *B. halotolerans* strain MSR-H4 and usage of both was highly efficient in the remediation of pendimethalin-polluted water. This means that *B. halotolerans* strain MSR-H4 increased the ability of *A. pinnata* 7001 to degrade of pendimethalin. Also, the results showed that the higher degradation rate of pendimethalin (96.66%) occurred by *B. halotolerans* strain MSR-H4 and *A. pinnata* 7001 together after 21 days of treatment. While in the controls, the rate of pendimethalin degradation slowly occurred; whereas the degradation percentages of pendimethalin were 12.5, 32.5 and 47.5% after 7, 14 and 21 days, respectively. The degradation of pendimethalin in the control may be attributed to hydrolysis and volatilization **(Wei *et al.*, 2001)**.

The degradation of pendimethalin by *B. halotolerans* strain MSR-H4 may be attributing to microbial activity to utilize of pendimethalin as the source of carbon or nitrogen or both. The *Bacillus* species have ability to degrade a wide variety of aromatic compounds in the natural environment; **Megadi *et al.* (2010)** found that *B. circulans* grow on pendimethalin (1000 mg/L) as the sole source of carbon. Also, **Wang, *et al.*, (2011)** found that a bacterial strain (HB-5) capable of mineralizing atrazine as a sole carbon and nitrogen sources for growth. Several researchers reported that microorganisms are highly active biological agent for biodegradation of pesticides in the environment **(Cho, *et al.*, 2008; Getenga, *et al.*, 2009; Govantes, *et al.*, 2009 and Tappin, *et al.*, 2012)**. The microorganisms

Table (1): Degradation percentage of pendimethalin at 400 mg/l in pendimethalin-polluted water by *Bacillus halotolerans* strain MSR-H4, *Azolla pinnata* and together after 7, 14 and 21 days of treatment

Treatments	Time after treatment (day)						LSD at	
	7 days		14 days		21 days			
	Concentration (mg/l)	Degradation %	Conc. (mg/l)	Degradation %	Conc. (mg/l)	Degradation %	0.05	0.01
Control*	350	12.50	270	32.50	210	47.50	10.50	15.92
***Bacillus halotolerans* strain MSR-H4**	210	40	110	59.25	80	61.90	6.82	10.33
Azolla pinnata	200	42.85	80	70.37	52	75.23	12.15	18.41
Bacillus halotolerans* strain MSR-H4 and *Azolla pinnata	50	85.71	20	92.59	7	96.66	2.62	3.98
LSD at 0.05	9.46		8.52		6.73		-	-
LSD at 0.01	13.76		12.40		9.80		-	-

***Control: pendimethalin-polluted water only**

can be degraded chemical pesticide either by using it directly as a source of nitrogen, carbon and phosphor **(Singh *et al.*, 2004, Belal *et al.*, 2008 and Awad *et al.*, 2011)** or co-metabolically **(Mallick *et al.*, 1999)**. In co-metabolism, the microorganism transformed of pesticide without using it or its constituent elements as a source of carbon and energy **(Alexander, 1999)**. Several microorganisms can be produce enzymes that are able to degrade pesticides and have therefore been suggested as suitable for bioremediation approach **(Entry *et al.*, 1996). Megadi *et al.* (2010)** showed that *B. circulans* bacterium degraded pendimethalin to 6-aminopendimethalin by nitroreductase and 3,4-dimethyl 2,6-dinitroaniline & pentane by oxidase, which was utilized as the source of carbon and energy

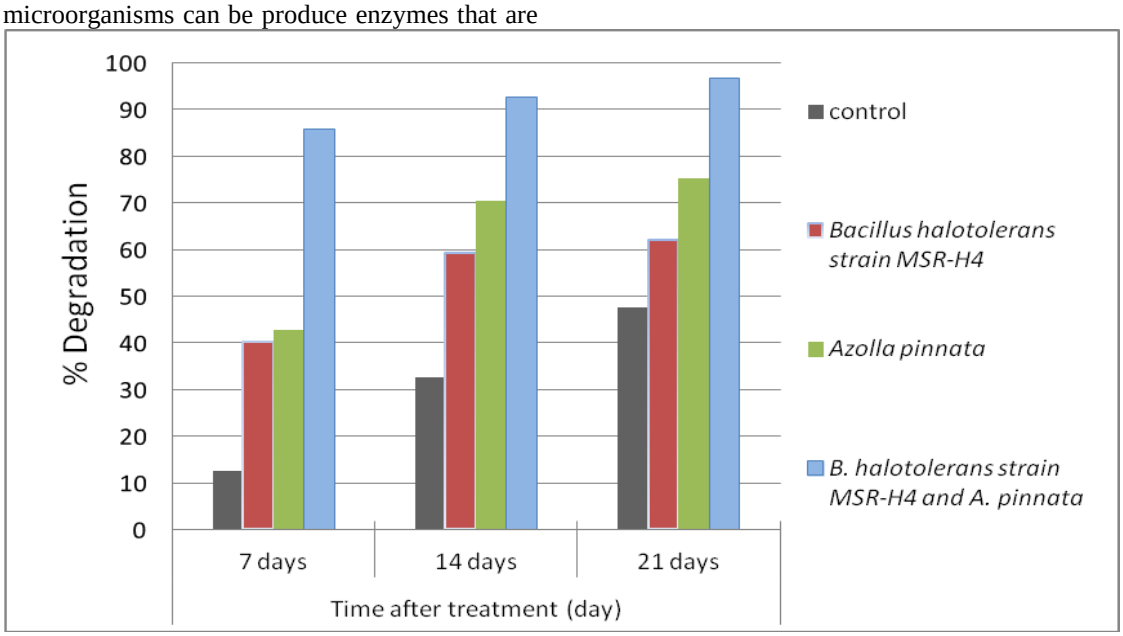

Fig. (3) % Degradation of pendimethalin at 400 mg/l in pendimethalin-polluted water by *Bacillus halotolerans* strain MSR-H4 and *Azolla pinnata* and together after 7, 14 and 21 days of treatment

for growth. Also, **Morel *et al.* (2015)** found that *Bacillus lehensis* degraded 93% of pendimmethalin at 1000 mg/l after 96 h and metabolized it to 6-aminopendimethalin and 3, 4-dimethyl 2, 6-dinitroaniline. Additionally, **Haiyan *et al.* (2016)** referred to *Bacillus subtilis* Y3 degraded 99.5% of 100 mg/L degraded 99.5% of 100 mg/l pendimethalin in batch liquid culture

within 2.5 days and utilized it as a sole carbon source and energy for growth.

The microbial degradation of pendimethalin has reported to occur most often by oxidative N-dealkylation and nitro-reduction **(Barua *et al*. 1990, Kole *et al.* 1994 and Megadi *et al.* 2010)**. The nitro group reduction and oxidative N-dealkylation decreased the herbicidal activity of pendimethalin, leading to its detoxification. Additionally, **Chirom *et al*. (2018)** showed that *Bacillus cereus* degraded more than 90% of 500 mg/l pendimethalin within 28 days of incubation time in the media inoculated with the bacterial isolate. **Yajie *et al.* (2019)** referred that *Clavispora lusitaniae* degraded 74% of 200 mg/l pendimethalin in liquid culture within 8 days.

Concerning phytoremediation, many researchers have been conducted widely in removal of pesticides from polluted water, such as **(Awuah *et al.*, 2004, Xia and Ma, 2006, Jamuna and Noorjahan, 2009). Xia and Ma (2006)** used *Eichhornia crassipes* as phytoremediation to remove malathion from polluted water. They found that *E. crassipes* degraded 56% of 10 ppm of malathion-polluted water. Also, **Dosnon-Olette *et al.* (2010)** found that *Lemna minor* and *Spirodela polyrhiza* were able to degrade of dimethomorph in agricultural waste water. Phytoremediation may be including pesticide uptake, metabolism inside the plant, or release of exudates leads to decrease of pollution **(McCutcheon and Schnoor 2003; Deepali *et al*.2009)**. Peroxidase enzymes may be playing main role in the oxidative metabolism of pesticides inside plants **(Roy *et al*. 1992)**.

The current our results indicated the great potential of *Bacillus halotolerans* strain MSR-H4 and *Azolla pinnata* 7001 in bioremediation of pendimethalin-polluted water.

The isolation and characterization of enzymes of *Bacillus halotolerans* strain MSR-H4 and *Azolla pinnata* 7001 needs further investigations in future work.

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المعالجة الحيوية للمياه الملوثة بمبيد الحشائش بنديميثالين

[*]إبراهيم سعيد إبراهيم - [**]إبراهيم متولى جمعة

* قسم وقاية النبات (مبيدات الآفات) – كلية الزراعة - جامعة الأزهر- مدينة نصر- القاهرة - مصر

**قسم البيئة والزراعة الحيوية– كلية الزراعة - جامعة الأزهر- مدينة نصر- القاهرة – مصر

الملخص العربى:

تم دراسة فعالية بكتريا باسيللس هالوتوليرانس ونبات الأزولا بيناتا بشكل منفصل (فردى) وهما معًا في معالجة المياه الملوثة بمبيد الحشائش بنديميثالين عند تركيز ٤٠٠ مجم / لتر. وكانت المعاملات على النحو التالي؛ مياه ملوثة بمبيد بنديميثالين (الكنترول)، مياه ملوثة بنديميثالين مع باسيللس هالوتوليرانس، مياه ملوثة بنديميثالين مع الأزولا بيناتا ومياه ملوثة بنديميثالين مع باسيللس هالوتوليرانس والأزولا بيناتا معاً. وقد تم تقدير نسب تحطم المبيد بعد ٧، ١٤ و ٢١ يوم من المعاملة باستخدام جهاز GLC. من خلال النتائج يمكن ترتيب فاعيلة المعاملات فى تحطم المبيد على النحو التالى باسيللس هالوتوليرانس والأزولا بيناتا معاً > الأزولا بيناتا > باسيللس هالوتوليرانس. وعلاوة على ذلك، وجد أن معدل التحطم زاد بزيادة وقت المعاملة. وكانت أعلى نسبة تحطم للمبيد ٩٦,٦٦٪ بعد ٢١ يوماً عندما تم استخدام باسيللس هالوتوليرانس والأزولا بيناتا معاً.