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Biodegradation of Atrazine Using Selected Marine Bacteria: Possibilities for Treating Pesticide - Contaminated Wastewater

Short Title: Atrazine Biodegradation using Marine Bacteria

ABSTRACT

The use of pesticides including atrazine can cause determinantal problems to the environment. Atrazine, 2-chloro-4-(ethylamine)-6- (isopropyl amine)-s-triazine, is one of the widely used herbicides**. In this work, thirteen** pure bacterial strains were isolated from water and sediments collected from different sites along the **Alexandria Mediterranean** Coast and were evaluated for their efficacy to biodegrade **Atrazine** at **three** elevated concentrations (**I:** 109, **II:** 299 & **III:** 438 mg/l) for 7 days. **Atrazine** residues were determined using gas chromatography (**GC**). Marine isolates exhibited very high atrazine biodegradation with removal efficacy ranging between 15.79**- 75.49**, **77.97- 97.13** and **27.4- 87.6%** at three elevated concentrations respectively. The results indicated that 5 of the isolates (**E7, 8, 9, 11** and **13)** were the most efficient, and active as Atrazine bio-degraders. They were affiliated as ***Bacillus pacificus*** strain **MCCC 1A06182** (**E7** and **E8**), ***Bacillus cereus*** strain **ATCC 14579** (**E9**) and ***Bacillus paramycoides*** strain **MCCC 1A04098** (**E11** and **E13**). The data obtained provided evidence indicating that the marine environment as a natural, rich and renewable source of bacteria with marvelous metabolic capabilities for efficient bioremediation of atrazine-contaminated aquatic environments or wastewater.

Key Words: Atrazine Biodegradation, Aquatic Media, *Bacillus*, Marine Ecosystem, Pesticide Pollution

1. INTRODUCTION

Around 30 to 60% of pesticides will reach the soil eventually end up in the soil **based on their use** **(Zhu et al., 2019; Ofaim et al., 2020)**. Pesticides and their metabolites have extremely drastic effects on human health as well as wildlife and the environment. The impact of pesticides in relation to the biological processes vary a lot and influence the food webs, soil, and aquatic organisms. The pesticides deleterious effects increase with increasing the concentration applied. The impact is not only and does not only kill the pest but causes a major disruption to the biological inhabitants of the soil leading to disruption to all soil functions **(Wirsching et al., 2020)**. This is attributed to the fact that the soil microbes and invertebrates have combined and critical effects to maintain soil functions, enhance food production and human health **(Brussaard, 2021)**. The biotic and abiotic processes result in the transformation of pesticides resulting in changes in their chemical composition. In addition to pesticide solubility and microbial population, the biodegradation of pesticides is highly influenced by various conditions present in the soil like: oil factors including pH, temperature, moisture, and the organic matter content **(Houjayfa et al., 2020)**.

Atrazine, 2-chloro-4-(ethylamine)-6-(isopropyl amine)-s-triazine, is one of the widely used herbicides although prohibited in the European Union in **2004** **(Billet et al., 2019)**. It is a non-polar toxic compound and considered as a serious environmental contaminant that pollutes water resources and soil worldwide due to its long-term use in crop production **(Jakinala et al., 2019; Li et al., 2019)**. Since it is attached to the soil by the polar soil colloids, it can end up contaminating ground water resources by being washed out from the root zone, particularly when applied prior to irrigation or heavy rainfall **(Carpio et al., 2021)**. Atrazine is used for the protection of major crops such as conifers, macadamia nuts, pineapples and chemical fallows. It is also used for industrial weed control especially during sorghum, corn production and sugarcane. It is applied as pre- and post-emergence herbicide **(El-Bestawy et al., 2013)**. Since it is both inexpensive and effective, it is suitable to the production systems which generates low profit. The environmental fate of Atrazine (half-life: 13-261 day) depends on many effectives including the attachment to soil particles, uptake, transport through the runoff and leaching and biodegradation. It was reported that the original applied compound and biodegradation metabolites were frequently often found in groundwater **(Espín et al., 2020)** and even in raw drinking water decades after application, sometimes at concentrations exceeding the maximum permissible limit of 3 mgL^{-1} according to the United States Environmental Protection Agency (USEPA) **(Qu et al., 2020)**.

Because of Atrazine persistence in soils and its runoff to surface and groundwater, deleterious environmental consequences have emerged, although it is less toxic to humans as compared to other chlorinated herbicides **(El-Bestawy et al., 2013; El-Bestawy et al., 2014)**. Such problems include reduced biodiversity and damaged future crops and food contamination

(Fernandes et al., 2020, Carpio et al., 2021). It has long-term reproductive and endocrine-disrupting effects, interrupts regular hormonal functions and causes defects in human birth, reproductive tumours, and weight loss in both humans and amphibians **(Ma et al., 2017)**. Atrazine leads to low birth weights, low sperm counts in men, menstrual problems and known as a probable human carcinogen **(Houjayfa et al., 2020; Zhang et al., 2019)**.

As such, the atrazine removal from the environment is considered imperative and is of growing public health concerns. Atrazine removal from contaminated soils, sediments, and water involves either microorganisms mediated biotic transformation processes **(He et al., 2019; Lihl et al., 2020),** or abiotic processes via photochemical and chemical reactions **(Rózsa et al., 2019; Shawky et al., 2020)**. However, bioremediation involving microbial communities is more effective and remains the most promising approach used for pesticide degradation **(Houjayfa et al., 2020; Wirsching et al., 2020)**. Due to the large-scale agricultural utilization in Saudi Arabia, especially in corn cultivation, and the dangerous atrazine toxic effects to both humans and the environment. For this reason, new microorganisms have been sought to completely degrade atrazine present in the environment. The marine environment is well known as a very attractive and rich natural resource for macro- and microorganisms with potent capabilities ranging from production of many bioactive compounds (antibiotics, anticancer and cardiovascular agents) to biodegradation of toxic environmental pollutants **(Fenical, 2020; Lyu et al., 2021 Zhu et al., 2021)**. Therefore, the present study aimed to explore the ability and efficiency of selected marine bacterial isolates to degrade atrazine. The outcome of this research is to effectively remediate and control the widespread atrazine pollution in the environment by using potent indigenous bacteria present in the soil.

2. MATERIALS & METHODS

2.1. Sampling

Water and sediment samples were collected from 4 chemically and biologically different sites along the **Alexandria Coast**, extending from **Abu-Qir** in the Far East through **Sidi Gaber, El-Selsela** until El- **El-Anfoushi** in the central part of the coast (**Fig. 1**). Abu-Qir, the most industrialized area in **Alexandria**, has a total area of about 38,000 hectares. It is a semi-circular, shallow water area with a depth ranging from less than one meter along the shore, increasing gradually to a maximum of 15 m around the middle **(Maged** and **Mikhail, 1990)**. **Sidi Gaber** and **El-Shatby** are used for swimming and recreational purposes as well as residential area **(El-Bestawy et al., 2011 & 2017)**. In the central part of **Alexandria Coast**, the **Eastern** and Western harbours as well as **El-Anfoushi** lie. They are shallow, protected embayment, semi enclosed circular basins. The four sites were selected based on type and extent of pollution prevailed from industrial (**Abu Qir**) and domestic (**Sidi Gaber, El-Selsela** and **El-Anfoushi**) discharges.

Fig. 1

Samples were collected according to specifications set by the **International Organization for Standardization (ISO 5667/6, 2016)** and **ISO 5667/10 (2020)**. Samples collection was carried out at a depth of 25-35 cm below the seawater surface 50 m off-shore in 250 mL-glass screw autoclaved capped bottles with wide mouthed openings. Special stainless steel sampling rod was used for this purpose. The bottles were opened at the time of collection. Sediment samples were collected using a piston corer at a depth of 20 cm and transferred sterilized plastic bags that were tightly closed. All samples were analysed in triplicates. Samples were maintained on ice in an ice box at 4 ºC while being transported to the laboratory and were processed within 2 to 3 h post collection. These samples were used to isolate bacteria based on the fact that microorganisms inhabiting polluted environments acquired high resistance, enzymatic, degradative capabilities and possess astonishing metabolic activities.

2.2. Isolation of Marine Bacteria

Marine bacteria were isolated from water and sediment samples on nutrient agar plates (prepared with filtered marine water) using pour plate technique of the standard plate count method **(Baird et al., 2017)**. Purification of heterotrophic bacterial isolates was performed using streaking method on nutrient agar plates (**NA, Oxoid, England**) and incubated at 37°C. After culturing and sub-culturing, thirteen pure isolates (designated **E 1-13**) were obtained. The purified isolates were inoculated onto **NA** slants, incubated as described earlier and then refrigerated for later use.

2.3. Atrazine Stock Solution

Atrazine (**2-chloro-4-(ethylamine)-6-(isopropyl amine)-s-triazine**) stock solution was prepared by dissolving technical Atrazine (80% active ingredient) in deionized water reaching a final concentration of 1,000 mgL^{-1}. Atrazine stock solution was sterilized by filtration using 0.22 μm polycarbonate membrane (manufacturer and address) to avoid precipitation or chemical changes during autoclaving **(Baird et al., 2017)**.

2.4. Synthetic Wastewater

Concentrated synthetic wastewater seeded with atrazine was used in bioremediation assays. It was prepared by dissolving the following chemical ingredients (g) in in one 1liter distilled water: **NaCl (40.7), $CaCl_2.2H_2O$** (0.37), **H_2MoO_4** (0.31) **Tripton** (122.1), **Na_2SO_4** (4.46), **$MnSO_4$** (0.57), **K_2HPO_4** (4.46), **$MgCl_2.6H_2O$** (0.37), and **NaOH** (0.08) **(O¨zbelge et al., 2005; El-Bestawy et al., 2013)**. After that, a one liter of the working wastewater was prepared by adding 10 mL concentrated synthetic wastewater to 990 mL distilled water and was autoclaved at 121°C for 20 min.

2.5. Bioremediation Bioassays

Pure marine bacterial isolates were investigated for the removal of atrazine in liquid culture from synthetic wastewater to be able to identify the most promising candidates. They were individually activated by transferring a loop full from each slant into 250 mL flask contained 200 ml nutrient broth (NB) and incubated at 37°C and 150 rpm shaking for 24 h. After activation, these inocula (200 mL each) were individually transferred into one-liter conical flasks containing 800 mL synthetic wastewater reaching a final volume of one liter (3 replicas each isolate) with definite aliquots of atrazine stock solution reaching elevated atrazine levels (**I**: 100, **II**: 250 & **III**: 500 mg/L) at pH 7. Immediately after inoculation of wastewater with the bacteria, all cultures were aseptically sampled and bacterial counts were taken at zero time point (data not shown). In addition to the inoculated wastewater (**39** cultures), three 1-L un-inoculated synthetic wastewater flasks were prepared to have the **3** atrazine levels and used as control. Also, all inoculated (treatment) and un-inoculated cultures were sampled immediately after inoculation to determine the start-up Atrazine concentration after which they were incubated at room temperature ≈ 25-30 °C since this experiment was performed during late spring. The experimental duration was 7 days and samples were aseptically collected at 24 h interval. Bacteria free wastewater (control) contained the same amount of atrazine were treated under to the same conditions as treatment cultures and used as a control to confirm the role of the tested bacteria. Fifty ml from each culture was drawn and residual levels of atrazine were assayed at each exposure time. Removal efficiencies of Atrazine by the tested bacteria were calculated to determine the efficacy of the remediation process and determine and identify the best degrading bacterial isolates (**Eq. 1**).

Removal Efficiency (RE %) = C0 - RC/ C0 X 100 …… Eq. 1 (El-Bestawy et al., 2020 & 2021)

Where **C0 =** Initial Concentration before Treatment (Zero Time);
RC= Residual Concentration after Treatment at each Exposure Time

2.6. Atrazine Residues Analysis

2.6.1. Extraction of Atrazine from Water Samples

Extraction of Atrazine from treated and untreated wastewater was done as previously described **(EL-Saeid** and **Alghamdi, 2020)**.

2.6.2. Extract Clean Up

The cleanup procedure of the extracted residue was done using a previously published method of **Wang S.-Y et al. (2020)**.

2.6.3. Gas liquid Chromatography Determination

Gas liquid chromatography was done as indicated in a previous publication of ours (**El-Bestawy et al., 2013, 2014 & 2017; EL-Saeid and Alghamdi, 2020**).

2.7. Atrazine Recovery Efficiency Study

2.7.1. Atrazine Recovery

To define the efficiency of the determination method for the recovery of atrazine, untreated samples of water were spiked with known quantities of Atrazine active ingredient solutions. Spiked samples were then undergone Atrazine extraction, cleaning-up and determination.

2.7.2. Preparation of Blank Solution

The used solvent and the anhydrous sodium sulphate in the fractioning and clean up were checked for purity of Atrazine and for the presence of any traces of the Atrazine before their use.

2.8. Molecular Characterization of Marine Bacteria

Five bacterial isolates out of the thirteen tested isolates showed the highest Atrazine biodegradation capability during biotreatment processes, therefore, they were molecularly characterized. Total genomic DNA was extracted from 5 mL overnight NB culture of the purified isolates according to the method described by **Sambrook et al., (1989)**. Then fragments of the 16S rDNA gene were amplified using the primers B341F (5'-CCTACGGGA GGCAGC), and 1392R (5'-ACGGGCGGTG TGTRC-3') as described by **Ausubel et al. (1999)**. Each of these purified products was sequenced and the resulting DNA sequences were phylogenetically analyzed using the BLAST search program (**Hall, 1999**).

2.9. Statistical Analysis

Mean (3 replicates) and standard error values were determined for all the parameters and the results were expressed as mean ± standard error. The data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison in order to compare treated groups with control. The differences were considered significant at $P < 0.05$ (95% of the confidence level). The data was analyzed using the Statistical Package for the Social Sciences program (SPSS) for windows (Version 20).

3. RESULTS & DISCUSSION

Atrazine was tested at elevated concentrations as enrichment and acclimatization approach to select the resistant isolates from one side as well as testing bacterial abilities for handling high levels of Atrazine to simulate situations during pollution accidents or disaster from the other.

3.1. Molecular Characterization of the Most Active Bacterial Isolates

The present study amid to evaluate the efficacy of thirteen marine bacterial isolates for atrazine degradation. Five of those isolates **(E 7, 8, 9, 11** and **13)** had the highest degradation capabilities even at the highest tested concentration. **Table 1** compiles Gen Bank accession numbers of the highest sequence similarity of most active atrazine degraders as well as the closest neighbor(s) to their 16S rDNA gene partial sequences. Sequences of the five isolates were affiliated to members of the genus ***Bacillus.*** Isolates **E 7** and **8** were identified as ***Bacillus pacificus*** strain **MCCC 1A06182** (100 and 99.85% sequence similarity) respectively**,** isolate **E 9** was ***Bacillus cereus*** strain **ATCC 14579** (similarity 99.9 %) while both isolates **E 11** and **13** were identified as ***Bacillus paramycoides*** strain **MCCC 1A04098** (98.44 and 99.16 % similarity) respectively (**Table 1**). The phylogenetic relationships of the experimental isolates and closely related species were analyzed using the multi-sequence alignment program (MEGA 5) was used to evaluate the phylogenic relationship of the experimental isolates and their closely related species and the data are shown in the phylogenic tree (**S1**) while their 16S ribosomal RNA partial sequences and alignments are presented in the supplementary material (**S2**)

Table 1, S1 & S2

It was reported that microorganisms such as ***Pseudomonas***, ***Bacillus*** and ***Arthrobacter*** are well known natural degraders to aromatic compounds like aromatic amino acids, phenols, or quinones **(Rotta et al., 2018; Kapoor and Saini, 2019; Kundu et al., 2019; Wang- Q et al., 2020)** prevailed in pharmaceuticals wastewater **(El-Bestawy et al., 2019)** where they have evolved catabolic pathways. Results of the present study confirmed the astonishing efficiency of tested ***Bacillus*** spp. for atrazine degradation and removal from contaminated wastewater which is consistent with and supported by many workers **(El-Bestawy et al., 2013 & 2014; Swapna et al., 2016; Khatoon** and **Rai, 2018; Jakinala, et al., 2019),** especially those previously exposed to the herbicide or its analogues **(Houjayfa et al., 2020; Li et al., 2019; Ma et al., 2017; Yang et al., 2018; Ye et al., 2016)**. Atrazine biodegradation was remarkably enhanced through bioaugmentation of exogenous potent atrazine degraders into contaminated media and biostimulation of the indigenous microorganisms **(Zhu et al., 2019; El-Bestawy et al., 2014; El-Bestawy and Zabermawi, 2017)**.

3.2. Atrazine Biodegradation at the Lowest Tested Concentration (I)

Biodegradation of Atrazine at the lowest tested concentration **I** (initial concentration: 109 mgL^{-1}) showed regular trend of decreasing the residual concentration (**RC**)with time reaching the lowest after 7 exposure days by all the tested bacteria except isolate 4 (after 3 exposure days) with no clear variations in its metabolic activity till the end of the experiment. As shown in **Table S3** and **Figure 2,** the highest **REs** % **(Removal Efficiency)** values of atrazine at concentration **I** (ranged between 15.79 and 75.49 %) were achieved by the tested bacteria at the last exposure day with relative variations among them. **REs** of 75.49, 75.41, 70.61 and 67.90 % were reached by isolates **E 9, 11, 6** and **8** respectively. Isolates **E 5, 3, 12, 4** and **13** recorded intermediate atrazine removals (62.77, 57.0, 51.18, 51.49 and 50.18 % respectively) compared to the other tested bacteria in the treatment duration. However, isolates **E 10, 1, 7** and **2** were the least active in atrazine biodegradation recording 47.83, 36.93, 33.09 and 21.23 % **RE** respectively. On the other hand, the control recorded very low atrazine removal (15.79 %) after 6 days confirming the active role of the tested bacteria towards atrazine biodegradation.

Table S3 & Fig. 2

3.3. Atrazine Biodegradation at the Intermediate Tested Concentration (II)

Atrazine **II** recorded 299.0±2.02 mg/l as the initial **IC** (**Table S2**). Biodegradation showed irregular removal trend by most of the tested cultures except for isolates **E 1, 2, 4, 8** and **13** that showed their highest **RCs** after one exposure day followed by regular decrease reaching their lowest **RCs** after 6 and 7 exposure days. At concentration **II,** the tested cultures including the control showed generally higher removal ranges of Atrazine (**Fig. 3**) compared to those obtained by the same cultures at the lowest concentration **I**. The highest achieved atrazine **REs** ranged between 77.97 and 97.13 %. **REs** of 97.13, 89.86, 87.80, 86.52 and 86.10 were achieved by isolates **E 8, 9, 11, 13** and **7** respectively. Other tested cultures reached considerable atrazine removal ranged between 77.97 % by **E 5** and 83.36 % by **E 1**. Surprisingly, at this concentration, control culture recorded considerably high removal range (65.52 % after 24 h to 88.44 % after 6 days).

Table S4 & Fig. 3

3.4. Atrazine Biodegradation at the Highest Tested Concentration (III)

Atrazine **III** recorded 438.8 mg/l as the **IC** (**Table S 5**). Atrazine biodegradation showed very clear and regular trend at this concentration with decreasing concentration with time reaching the lowest **RCs** after 7 exposure days. Unexpectedly, very high removals exceeding 85% were obtained by some of the tested culture at this very high atrazine level. The highest atrazine **REs** ranged between 27.4 and 87.6 % were achieved by **E 7, 13** and **11** (**87.6, 85.4** and **82.8** % respectively). Seven isolates (**E 12, 5, 10, 9, 8, 6** and **2**) had intermediate **REs** (**75.8, 73.8, 73.0, 71.1, 64.8, 57.3** and **56.5** % respectively) compared to the other tested bacteria. While isolates **E 1, 4** and **3** reached **45.8, 31.8** and **27.4%** respectively that considered relatively the minimum achievements of the highest atrazine **RE** range (**Fig. 4**). Active atrazine degrading cultures at this concentration considered highly resistant against its toxicity and possess all the required enzymes for its biodegradation. However, as expected the control (unseeded wastewater) showed the least removal range (**8.4-18.4** %) after **1** and **3** exposure days. The lowest atrazine **RCs** in the treated wastewater recorded **26.7, 8.6** and **63.9** mgL^{-1} achieved by isolates **E 9, 8** and **13** at **I**, **II** and **III** Atrazine concentrations tested respectively. Such **RCs** are much higher than the maximum permissible limit (**MPL**) of Atrazine (≤ 0.1 mgL^{-1}) because Atrazine initial concentrations tested in the present study were also very high. This limit is set by environmental laws in **Egypt** and **Saudi Arabia** to protect the aquatic life from any ecological disturbances and from hazardous discharges from the soil environments. However, the five promising Atrazine degraders could be immobilized using any supporting media and used as a continuous treatment system as individual or serial units.

Table S5 & Fig. 4

Statistical analysis (**Table 2**) revealed that at the first atrazine concentration isolate **E 9** is the most effective for atrazine degradation and significantly ($P < 0.05$) different compared to the control and other tested cultures. Values denoted by different letters within same column represent significant differences ($P<0.05$). There was a significant decrease in atrazine concentration after treatment by all the tested strains when compared to the control. Wastewater treated with isolates **E 6, 8, 9** and **11** showed significant decrease in atrazine concentration compared to other isolates confirming that such isolates have high atrazine removal efficiency rather than other isolates. At the intermediate atrazine concentration, there was a significant ($P<0.05$) decrease in atrazine concentration after treatment using isolates **E 4, 8, 9** and **13** compared to the control and all other tested groups but treated groups of isolates **E 1, 2, 3, 5, 7, 10** and **12** showed significant increase in atrazine concentration as compared to the control groups. On the other hand, the treated groups of isolates **E 6** and **11** showed insignificant changes in comparison to the control. Finally, at the highest tested atrazine concentration, groups treated with isolates **E 7, 11** and **13** showed significant ($P<0.05$) decrease in atrazine concentration as compared to all other treated groups. Moreover, there was a significant decrease in atrazine concentration in all the treated groups (by different isolates) compared to the control group.

Figure 5 (A-C) represents example **GC** chromatograms of Atrazine biodegradation at the three tested concentrations where average recovery from spiked samples recorded 89%. Biodegradation results of atrazine by the **13** tested isolates at the tested elevated concentrations concluded that 5 isolates considered the most resistant, efficient and active in the removal of the target herbicide, atrazine. They are isolates **7, 8, 9, 11** and **13**. Therefore, they were molecularly identified.

Figure 5 (A-C)

Batch treatment demonstrated a basic trend of increasing atrazine degradation in relation to increasing exposure time. Five strains belonged to **3** ***Bacillus*** spp. **(*B. pacificus, B. cereus*** and ***B. paramycoides*)** exhibited very high atrazine biodegradation ability with the highest achieved atrazine removal ranges recorded **15.79- 75.49**, **77.97- 97.13,** and **27.4- 87.6**% at **109, 299** and **438** mgL^{-1} atrazine initial concentration respectively at room temperature without shacking. These results indicated that these strains could utilize atrazine as a sole carbon, nitrogen and energy source with remarkably higher capability compared to other workers. For example ***Citricoccus* sp.** strain **TT3** could remove **50** L^{-1} atrazine in **66** h with 1% inoculum at **30°C** and pH **7.0** (Yang et al., 2018), ***Ensifer*** sp. isolated from an industrial soil can metabolize 100 mg L^{-1} atrazine completely within 30 h Ma et al., 2017), ***Shewanella* sp.** **YJY4** from cornfield soil degraded atrazine (100 mg/l) to cyanuric acid completely after 36 h (Ye et al., 2016) and ***Rhodococcus*** sp. **BCH2** isolated from soil, long-term treated with atrazine was able of achieving a degradation level of 75 % atrazine (100 ppm) in liquid medium kept in the dark for 7 days at pH 7 and under aerobic conditions (Khatoon and Rai, 2018). These examples clearly confirmed the superiority of marine ***Bacillus*** spp. isolated during the present study for degradation of Atrazine reaching as high as **75.49**, **97.13** and **87.6**% at atrazine initial concentrations of 109, 299 and 438 mgL^{-1} respectively in 7 days. As shown here the peak **REs** of Atrazine were achieved at concentration **II** and continued to **III** (97.13 and 87.6% respectively). This may be attributed to the stimulation in their metabolic activity and induction of the required degradation enzymes with increasing atrazine concentration from **I** to **II** and **III** (almost 3 and 4 folds respectively) by the resistant and atrazine degrading isolates. This is confirmed by atrazine removal range at concentration **III** (27.4 and 87.6 %) where 3 resistant isolates **E 7, 13** and **11** could achieve 87.6, 85.4 and 82.8 % respectively which considered remarkable removal at such high initial concentration tested.

Moreover, such degradation efficiencies were achieved at ambient temperature without any other requirements or adjustment (i.e. pH, agitation, temperature, carbon and nitrogen amendments…etc.) as with other workers (Yang et al., 2018; Ma et al., 2017; Khatoon and Rai, 2018) which make the present selection even better for decontamination of open environments as well as industrial wastewater. However, the lowest atrazine **RCs** in the treated wastewater (26.7, 8.6 and 63.9 mgL^{-1} at **I, II** and **III** tested Atrazine concentrations respectively) were not compatible with the **MPL** set by environmental regulation. But this problem can be solved reaching acceptable limits for safe discharge by immobilization of the promising Atrazine ***Bacillus*** spp. degraders forming biofilm on or in suitable carriers to be used as a continuous treatment system in individual or serial units. This suggestion is supported by **Khatoon** and **Rai (2018)** who reported that **α-Fe_2O_3** immobilized ***Bacilli*** cells degraded atrazine at a wide range of physicochemical factors (temperature: 20 to 45°C, and pH: 4.0 to 9.0, Atrazine concentration: 50 to 300 mgL^{-1} and stirring speed: 50 to 300 rpm). This illustrates that ***Bacilli*** cells modified by fixation as biofilm or decorated with specific nanoparticles such as **α-Fe_2O_3** could tolerate a higher range of Atrazine concentration compared to their free cells. **The** data obtained in this study will assist to effectively remediate and control the widespread of atrazine and possibly other pesticides in the environment.

4. CONCLUSION

Atrazine biodegradation using **thirteen** pure marine bacterial isolates concluded the following points:

1. Among the thirteen isolates, five {***Bacillus pacificus*** (**E7** and **E8**), ***Bacillus cereus*** (**E9**) and ***Bacillus paramycoides*** (**E11** and **E13**)} were found to be the most effective, efficient and active in the degradation and removal of the target herbicide.
2. Atrazine removal ranged between **15.79- 75.49**, **77.97- 97.13** and **27.4- 87.6%** equivalent to **26.7, 8.6** and **63.9** mgL^{-1} were achieved by the tested bacteria at Atrazine concentrations **I, II**

and **III** (109, 299 & 438 mgL^{-1}) respectively. Atrazine residues are still much higher than the maximum permissible limit (**MPL**) of Atrazine (≤0.1 mgL^{-1}).

3. To reach acceptable limits for safe discharge, Atrazine degraders can be immobilized and used in a continuous treatment system as individual or serial units.

4. Achieved results provide an excellent potential for manipulating marine environment as a natural, rich and renewable source for bacteria with marvelous metabolic capabilities for efficient atrazine biodegradation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fig. 1. Alexandria Coast and the four sampling sites (marked yellow)

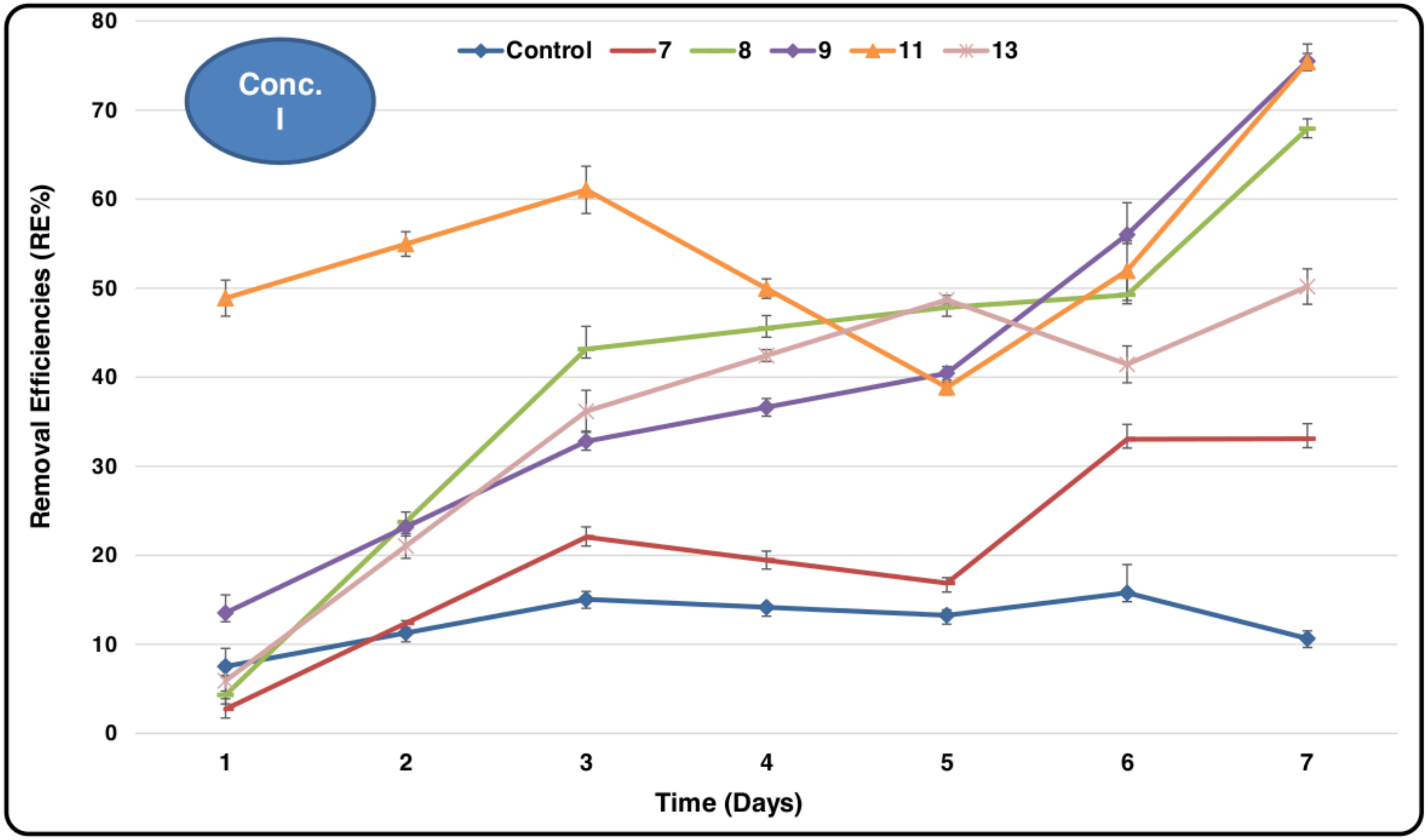


Fig. 2: Removal efficiencies (%) of Atrazine at the lowest tested concentration using the most efficient marine bacterial isolates for 7 exposure days

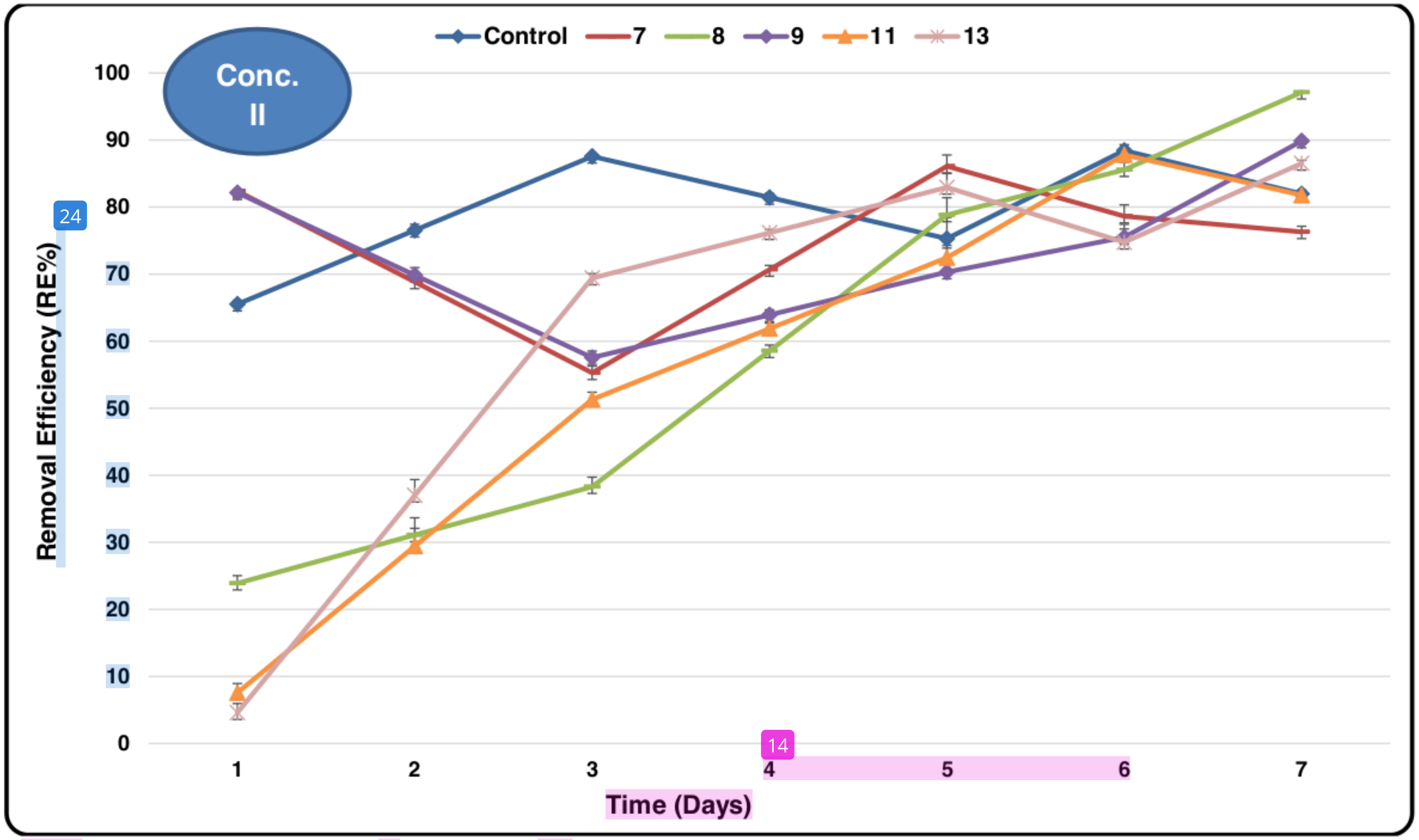


Fig. 3: **Removal efficiencies (%) of Atrazine at the intermediate tested concentration using the most efficient marine bacterial isolates for 7 exposure days**

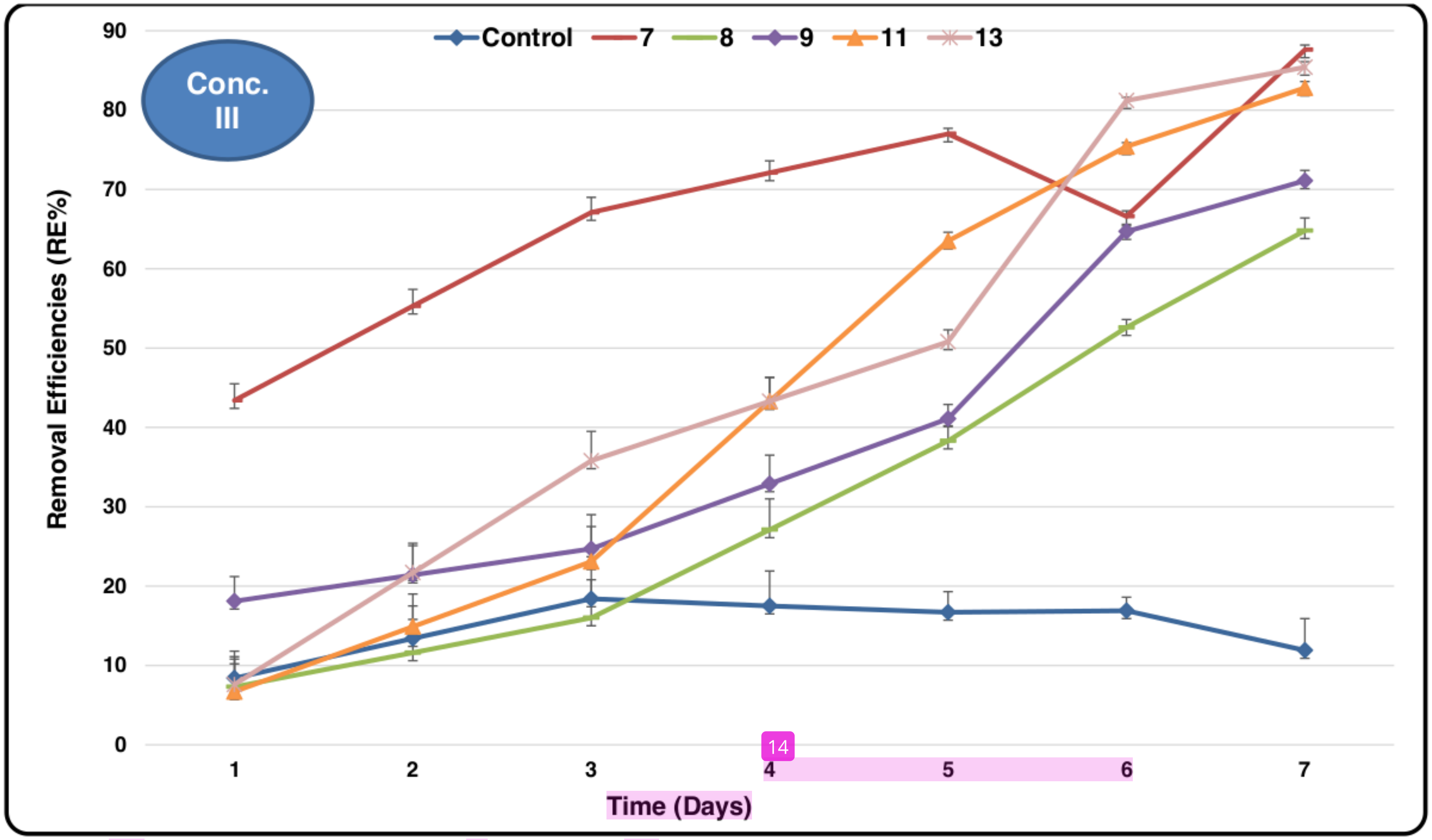


Fig. 4: Removal efficiencies (%) of Atrazine at the highest tested concentrations using the most efficient marine bacterial isolates for 7 exposure days

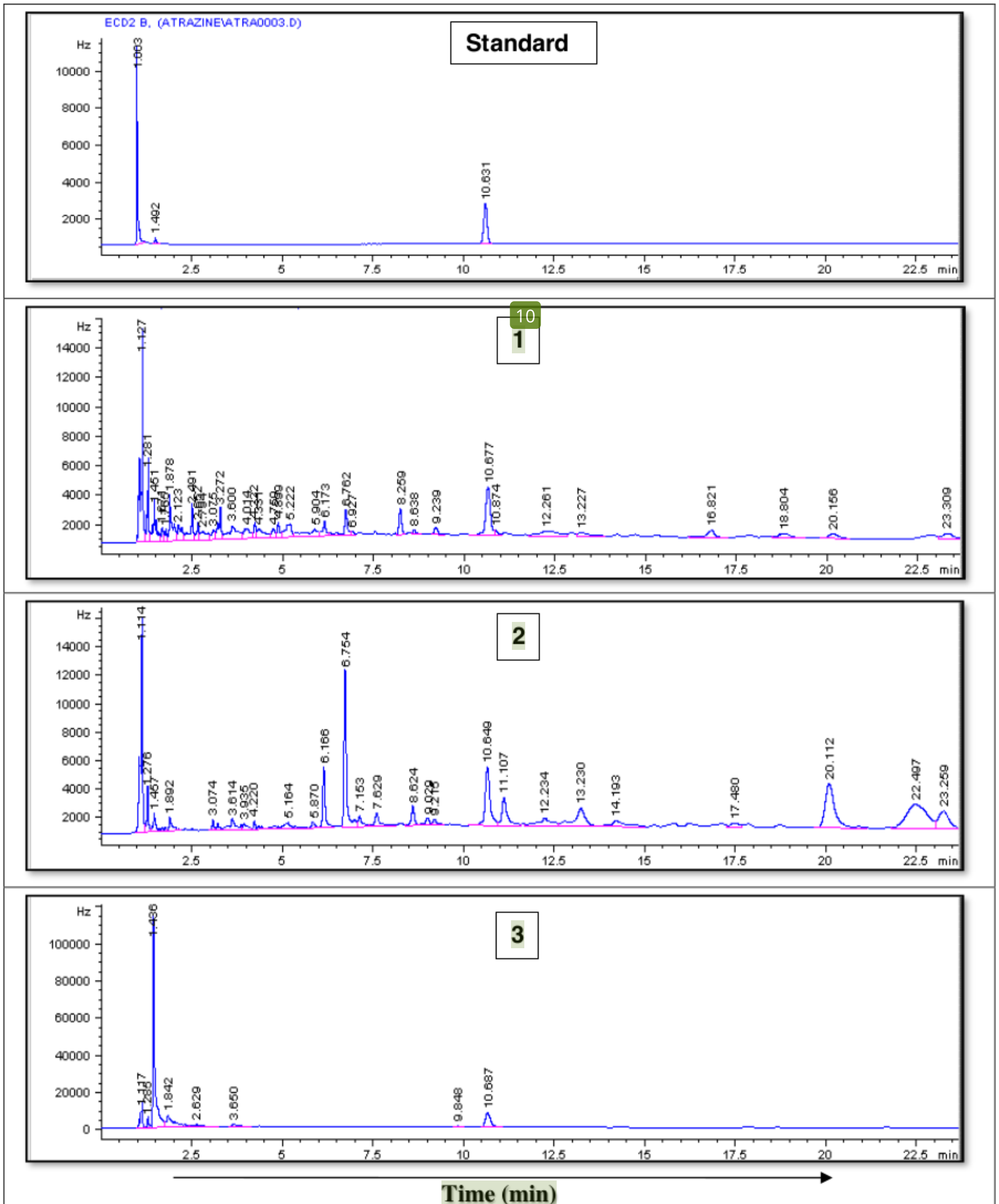


Fig. 5 (A): Concentration I

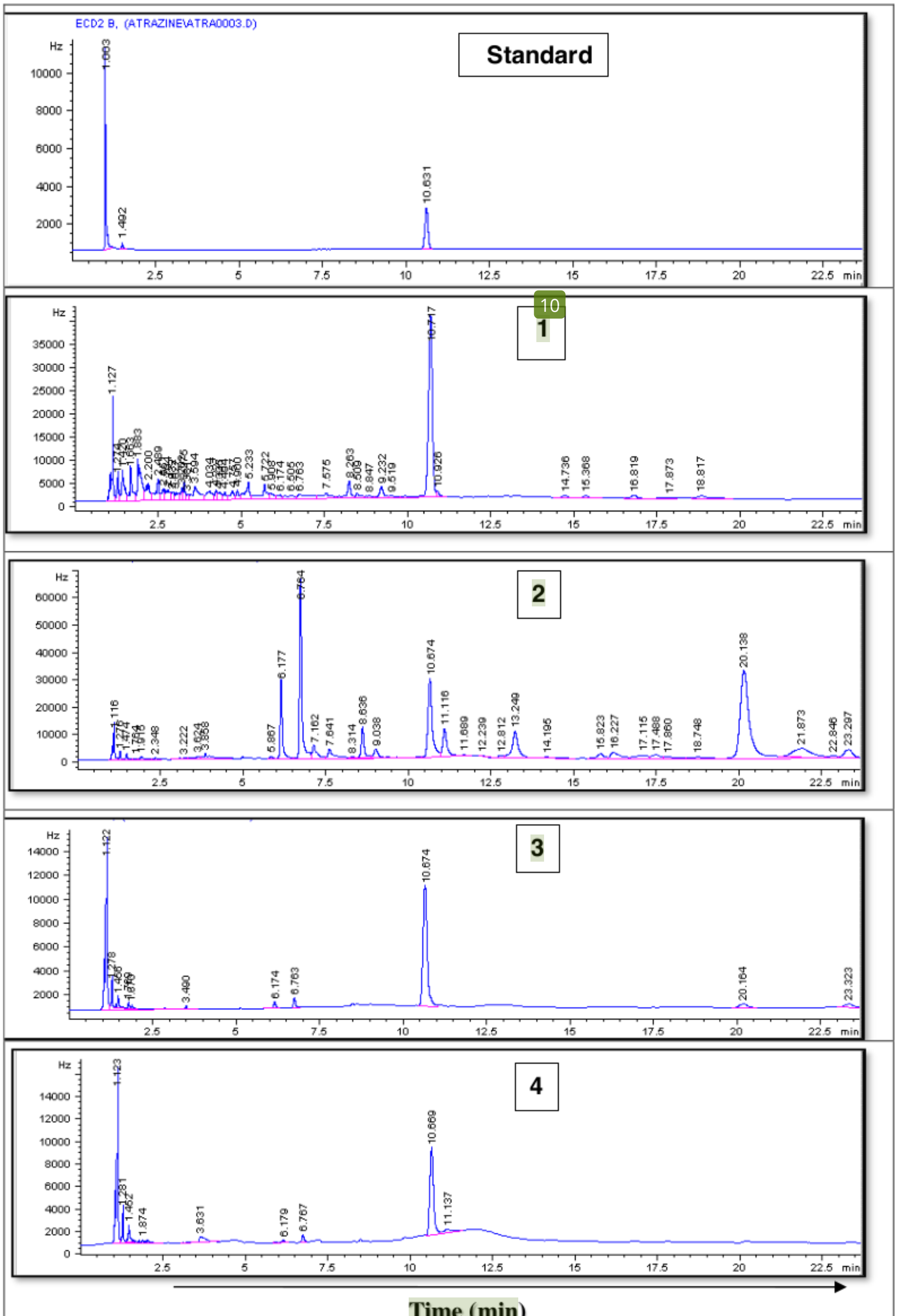
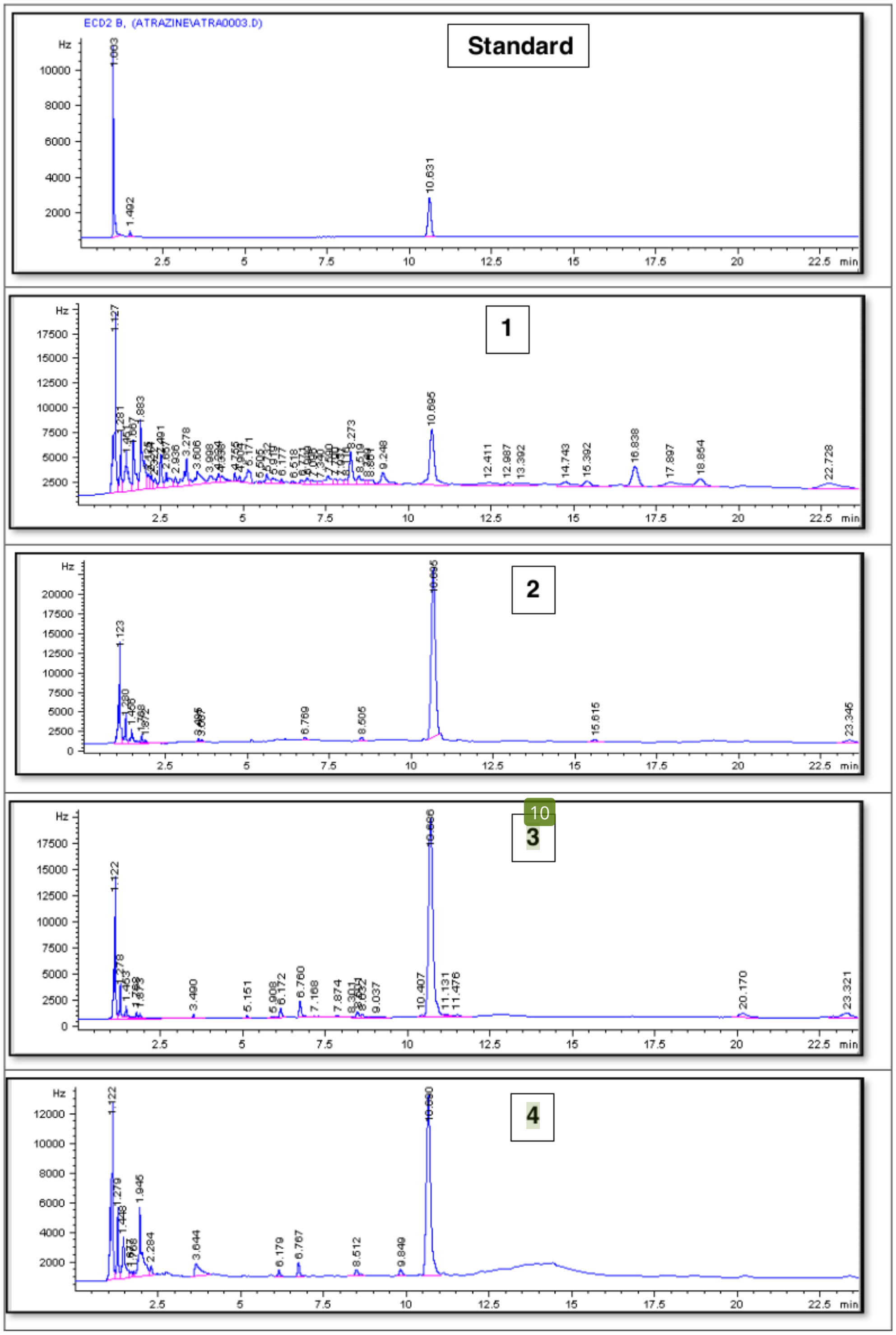


Fig. 5 (B): Concentration II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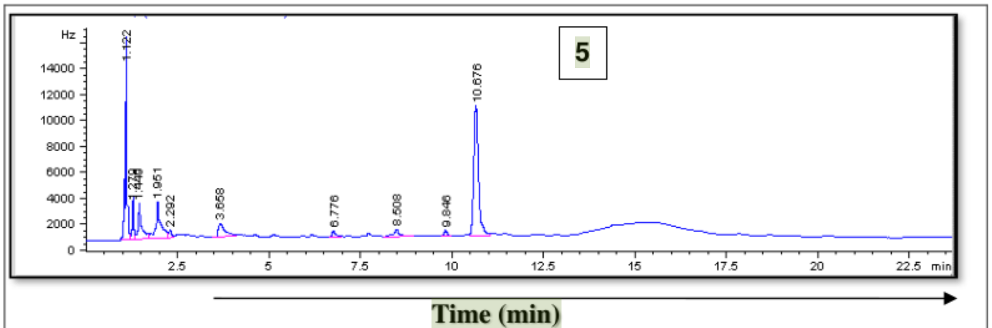

Fig. 5 (C): Concentration III

Fig. 5: GC Chromatograms of Atrazine residues after rreatment using A) *Bacillus paramycoides* strain MCCC 1A04098 at the lowest tested concentration after 1) 3 days, 2) 5 days and 3) 7 days, B) *Bacillus pacificus* strain MCCC 1A06182 at the intermediate tested concentration after 1) 2 days, 2) 3 days, 3) 5 days and 4) 6 days and C) *Bacillus cereus* strain ATCC 14579 at the highest tested concentration after 1) 2 days, 2) 3 days, 3) 5 days, 4) 6 days and 5) 7 days

Table 1: Similarity percentages to the nearest neighbors of the selected isolates

Isolate No.	Nearest Neighbor(s)	Gen Bank accession of the Nearest Neighbor	Similarity %
7	*Bacillus pacificus* strain MCCC 1A06182	NR157733.1	100
8	*Bacillus pacificus* strain MCCC1A06182	NR157733.1	99.85
9	*Bacillus cereus* strain ATCC 14579	NR074540.1	99.9
11	*Bacillus paramycoides strain* MCCC 1A04098	NR157734.1	98.44
13	*Bacillus paramycoides strain* MCCC 1A04098	NR157734.1	99.16

Table 2: Statistical variations in Atrazine biodegradation among the different tested bacteria

Isolate	Concentration		
	I	II	III
Control	97.4±0.57^{a}	54.05±0.57^{e}	387.71±4.0^{a}
1	68.8±0.63^{c}	65.07± 0.63^{c}	239.28±2.4^{d}
2	85.9±0.66^{b}	58.90±0.66^{d}	191.25±1.9^{e}
3	46.9±0.54^{ef}	60.25±0.54^{d}	319.71±3.3^{b}
4	68.3±0.52^{c}	50.13±0.52^{f}	300.06±3.1c
5	40.6±0.98^{fg}	82.21±0.98^{a}	115.33±1.2^{gh}
6	32±0.63^{gh}	53.03±0.63^{ef}	187.94±1.9^{e}
7	72.9±0.84^{bc}	71.04±0.84^{b}	54.56±0.6^{j}
8	35±0.12^{gh}	8.60±0.12^{i}	154.74±1.6^{f}
9	26.7±0.35^{h}	30.42±0.35^{h}	127.16±1.3^{g}
10	56.9±0.69^{d}	58.74±0.69^{d}	118.74±1.2^{gh}
11	26.8±0.64^{h}	54.65±0.64^{e}	75.82±0.8^{i}
12	53.2±0.73^{de}	61.61±0.73^{d}	106.60±1.1^{h}
13	54.3±0.46^{de}	40.43±0.46^{g}	64.08±0.7^{ij}

Results are Expressed as Mean of 3 Replicates ± SE
RC mean values denoted by different letters (a-j) within same column represent significant differences (at $P<0.05$)
Means with the same letters are not statistically significant

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