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Investigating the Impact of Climate Change on Frequency of Atrazine-Degrading Bacteria in Karun River Sediments

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The use of pesticides and herbicides as a means of satisfying the nutritional needs of the growing world's population has been extensively increased. On the other hand however, it has led to entry of a large amount of pollutants in soil and water resources: a serious threat to the environment. This study has focused on the seasonal biodegradation capability of atrazine herbicide, which can be found in Karun River sediments due to significant use in sugarcane farms. At first, sampling was conducted in both summer and autumn at the depth of 3 to 5 cm of river sediments. After enrichment, atrazine-degrading aerobic bacteria were isolated and identified by biochemical tests, and the results of two seasons were compared. Eight bacterial strains including *Achromobacter insolitus*, *Delftia tsuruhatensis*, *Klebsiella pneumonia*, *Enterobacter ludvigjii*, *Serratia marcescens*, *Enterobacter* sp., *Bacillus* sp., and *Exiguobacterium profundum* were identified. Based on the statistical analysis using SPSS software, ANOVA analysis, and Duncan test, a significant difference has been observed between the results of two seasons, the counted bacteria in summer showed to be 4.965±0.016 cfu/ml, larger compared with 4.894±0.017 cfu/ml in autumn. Most of atrazine-degrading bacteria were Gram-negative. The percentage of Gram-negative and Gram-positive bacteria in summer was determined 77% and 23% respectively, whereas no Gram-positive bacterium was found in autumn. Seasonal temperature change showed a significant impact on the number of bacteria in both seasons. The final results also indicated that the identified bacteria are effective selections for atrazine bioremediation in the environment.

Key words: Biodegradation, Atrazine, Seasonal impact, Karun River sediments

Xenobiotic pollution of agricultural soil, surface and subsurface waters is one of the most essential issues of environmental protection in current conditions. Pollution by various pesticides involves extensive areas, due to their widespread and in many cases irresponsible use[1].

S-Triazine herbicides such as simazine and atrazine have been significantly used for controlling leaf and grassy weeds[2,3]. Atrazine (2-chloro-4-ethylamino-6-isopropyl amino-1,3,5-triazine) is a member of chlorinated s-triazine family, which has benzene-like aromatic structure with moderate mobility, high persistence in soil, and relatively high water solubility (33 mg/l) at the temperature 20°c . It is used to control of broadleaf and grassy weeds in corn, pineapple, sorghum, cotton and other crops as a selective herbicide, and in non-cropped industrial and fallow lands as a nonselective herbicide[4,5]. Thus it can be found in the atmosphere, surface/ground water[6].

According to Iran Plant Protection Organization (PPO) statistics, atrazine consumption has been reported about 250 tons in 2007, and its average consumption has been recorded up to 5 kg/ha[7].

There are various methods for atrazine removal including chemical oxidation, adsorption, hydraulic destruction, volatilization, phytoremediation, incineration and biodegradation.

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Biodegradation by microorganisms is the main mechanism of atrazine removal among natural physicochemical dissipation ways of s-triazines, and bioremediation has been reported a successful strategy for s-triazines removal in soil, since it is the only method by which atrazine can be completely mineralized[3,8]. Atrazine can be degraded mainly through biological processes such as N-dealkylation, dechlorination, and ring cleavage[9].

Atrazine is a relatively poor source of carbon and energy, but an excellent potential source of nitrogen[10]. Nitrogen released from atrazine metabolism acts as an energy source for the atrazine-degrading bacteria[11].

The isolation of various fungi and bacteria has been reported by researchers. According to their findings, dealkylate and dechlorinate atrazine, a variety of atrazine-mineralizing bacteria including members of the genera *Pseudomonas, Rhizobium*, *Acinetobacter*, and *Agrobacterium* have been so far isolated from atrazine-contaminated soil[8]. Atrazine degradation could also occur by means of *Arthrobacter* sp., *Chelatobacter heintzii*, *Rhodococcus* sp., *Acinetobacter* sp., *Streptomyces* sp., *Pseudomonas aeruginosa*, *Clavibacter michiganense*, *Enterobacter cloacae*, *Bacillus megaterium*, *Alcaligenes faecalis*, *Klebsiella ornithinolytica*, and *Agrobacterium tumefacien*[5,12].

Most of catabolic activity in bacterial cultures is observed between the temperatures of 20° to 30° C. Biodegradation efficiency is reduced at lower temperatures and it fully stops at temperatures below 15° C to 7 ° C. In some studies, the appropriate pH value for atrazine decomposition has been generally specified between 7 to 9[13]. In some other sources however, optimum pH value has been listed between 5.5 and 5.8, and pH values less than 6.5 have been suggested as a biodegradation inhibition factor[1].

Despite the lack of enough evidence about atrazine carcinogenesis, it has raised as a potential carcinogen for human being[4,14]. Atrazine toxicity has been reported low for human, but prolonged and frequent contact with this material can cause kidney, pulmonary and endocrine damages[1].

Radoservich *et al*. (1995) isolated an atrazine-degrading bacterial culture from an agricultural soil previously impacted by herbicide spills. That organism was able to use atrazine under aerobic conditions as the sole source of carbon and nitrogen[2]. Isolation and characterization of atrazine-degrading bacteria was studied by Cai *et al*. in 2003, with the aim of identification of suitable bioremediation candidates for environments contaminated with atrazine[15]. In 2007, Dehghani *et al*. studied several agricultural fields with a long history of atrazine use in Fars province, Iran, to investigate capability of atrazine biodegradation, which led to enriched culture with 88% capability of atrazine degradation. Seven Gram-negative and one Gram-positive bacterial strain were isolated from the soil at a corn field, which used atrazine as a sole source of nitrogen[14].

Karun River is one of the important aquatic ecosystems that have been affected by entry of different wastewaters. Today with increasing population and subsequently, increasing industrial, agricultural and urban wastewater, the river has more potential for being contaminated, which it would be an environmental risk to aquatic life of the region. Entry and accumulation of pollutants in the body of aquatic organisms and would endanger the human health as well[16]. There are a considerable number of sugarcane fields in Khuzestan province, facing with the danger of contamination by agricultural chemicals including atrazine. Nevertheless, an apparent lack of studies on atrazine biodegradation by bacteria in the contaminated river sediments can be observed. There is also no noticeable investigation into seasonal changes impact on the act of atrazine degradation bacteria with bioremediation objective. Therefore in the current research, atrazine biodegradation ability of isolated aerobic bacteria was investigated in summer and autumn after sampling from Karun River sediments, and the effect of season change on the diversity of bacterial strains was studied.

MATERIALS AND METHODS

Sampling

In the present study, sampling was conducted in both summer and autumn from sediments at the depth 3 to 5 cm, at three stations A, B and C, located in the Karun River. Each station was sampled three times, and the samples were

transported to the laboratory on ice, placed in sterile containers. Geographical coordinates of sampling locations (table 1) were determined by GPS devices.

Determination of pH and temperature

Water and sediment temperature of Karun River in both seasons was determined by means of thermometer at sampling stations. After sampling and transferring samples to the lab, water and sediment pH was also measured using pH meter and pH bar. The recorded parameters have been shown in Table 2.

Counting bacteria

The viable plate count method was used to counting bacteria. The collected sediment samples were diluted 10^{-1}–10^{-9}. The diluted samples were then cultured on agar nutrient with and without atrazine, by surface plate method. The cultured plates were incubated, and the colonies were eventually counted after 24 hours[17,18].

Enrichment and isolation of bacteria

Enrichment was performed using a basal salt medium (BSM) containing $MgSO_4.7\ H_2O$ (0.5 g), K_2HPO_4 (0.5 g), $FeCl_3.H_2O$ (10 mg), $CaCl_2.H_2O$ (10 mg), $MnCl_2$ (0.1 mg), $ZnSO_4$ (0.01 mg) and sodium citrate (0.01 mg)[12]. 95 ml of BSM containing 30 mg/l of atrazine was mixed with 5 grams of sediment sample. After one week, 1 ml of the mixture was again inoculated in 95 ml of BSM containing 30 mg/l of atrazine. Consequently, the final specimen was placed in an incubator shaker for one week, with the aeration of 30°C. The passage was repeated until the observation of turbidity caused by bacteria growth. The specimens were then cultured by sterile swab, and atrazine-resistant bacterial colonies were purified on blood agar medium[14].

Identification of atrazine-degrading bacteria

A number of identification methods including colony morphology, Gram stain, microscopic form and some biochemical tests including oxidase, catalase, TSI, urease, citrate, PD, OD, LD, MRVP and SIM were used to identify the isolated strains[19].

Statistical analysis

Comparison was made to determine the influence of season and location changes on the number of atrazine-degrading bacteria using SPSS statistical software and the analysis of variance (ANOVA).

RESULTS

In the current research, a total of eight aerobic atrazine-degrading bacteria were isolated from Karun River sediments. In the summer, two Gram-positive and six Gram-negative bacteria including *Exiguobacterium profundum*, *Bacillus* sp. and *Achromobacter insolitus*, *Delftia tsuruhatensis*, *Klebsiella pneumonia*, *Enterobacter ludwigii*, *Serratia marcescens*, *Enterobacter* sp. were identified respectively. In contrast, just five Gram-negative bacteria including *Achromobacter insolitus*, *Delftia tsuruhatensis*, *Klebsiella pneumonia*, *Enterobacter ludwigii* and *Serratia marcescens* were isolated in the autumn. According to the results of ANOVA analysis and Duncan test, a significant difference at p-value of 1% was observed between the results of two seasons. The impact of season, group, station, repetition, dilution and interaction effects of group on station and station on repetition was also recorded at p-value of 1%. The interaction effects of season on station, season on repetition and season on dilution were also of a significant difference of 5%. As shown in Figure 1, the average counted bacteria in summer was larger than autumn with 4.965 ±0.016 cfu/ml compared with 4.894±0.017 cfu/ml and a significant difference at the p-value

Table 1. Geographical coordinates of the sampling stations

Station	A	B	C
East	48° 39' 55,84"	48° 41' 12,87"	48° 41' 12,43"
North	31° 19' 04,62"	31° 20' 08,72"	31° 20' 07,68"

Table 2. Measured temperature and pH

Season	pH	Temperature
Summer	7.3	28
Autumn	7.1	20

of 1% was observed between the results of two seasons.

According to Figure 2, comparison can be made in average amount of counted bacteria. The amount of bacteria belonged to the group without atrazine is more than the group with atrazine, with the average of 5.033±0.015 cfu/ml compared with 4.825±0.018 cfu/ml. A significant difference at p-value of 1% can be seen between these two groups. The maximum and minimum counted bacteria between different repetitions were recorded in the first repetition with the amount of 4.991±0.019 cfu/ml and 4.830±0.026 cfu/ml. A significant difference at p-value of 5% based on Duncan test could be seen between all groups. Bacteria amount in the summer, either in the presence or absence of atrazine was estimated greater than those in autumn. There was a significant difference at p-value of 1% between these two groups.

In terms of station, the minimum and maximum frequencies of bacteria at station A and station B were respectively recorded 4.904±0.020 cfu/ml and 4.978±0.018 cfu/ml. All groups showed a significant different at the 5% p-value by Duncan test. Bacteria amount in summer at stations B, C and A was respectively has been measured 5.048±0.024, 4.939±0.029 and 4.907±0.027 cfu/ml. In autumn, the greatest amount of bacteria was

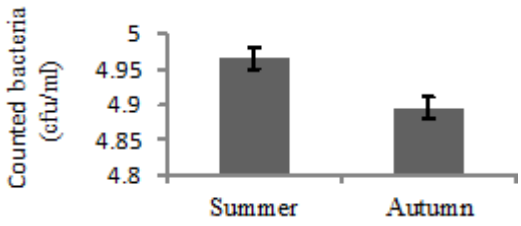

Fig. 1. Average counted bacteria in different seasons

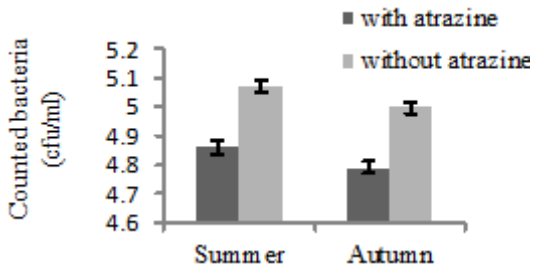

Fig. 2. Average counted bacteria in two seasons

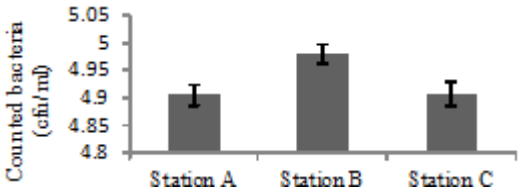

Fig. 3. Average counted bacteria in different stations

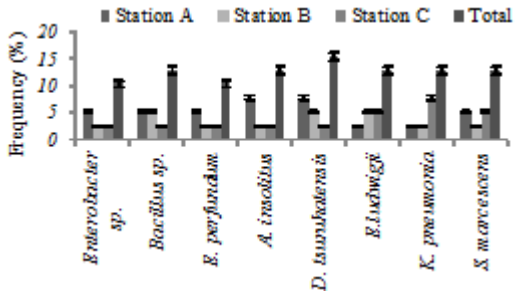

Fig. 4. Frequency percentage of isolated bacterial strains at different stations in summer

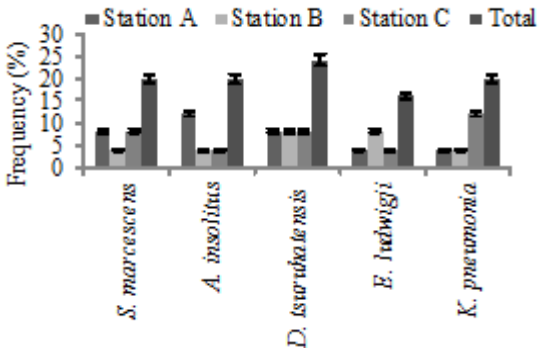

Fig. 5. Frequency percentage of isolated bacterial strains at different stations in auntumn

estimated at station B to the extent of 4.908±0.026, followed by station A and C with 4.900±0.026 and 4.873±0.029 cfu/ml (Fig. 3).

In addition, the number of Gram-positive bacteria was less than Gram-negative ones, with 23% in comparison with 77%. There was also a significant difference at the p-value of 1% between two above-mentioned groups. *Delftia tsuruhatensis* and *Enterobacter Sp*. with respectively with 15.4% and 10.3% had the maximum and minimum abundance percentages at all three stations in the summer (Fig. 4). Moreover, all isolated bacteria (100%) were Gram-negative at different stations in autumn. A significant difference was observed at the p-value of 1% between the Gram-negative and Gram-positive bacteria groups. In contrast, most of identified bacteria in various repetitions of all three stations belonged to *Delftia tsuruhatensis* with the percentage of 24% in the autumn, and the other bacteria accounted for 20% of the total population (Fig. 5).

DISCUSSION

Originally, pesticides have been developed as a means of supplying more food and nutritional needs for growing world's population. Even so, as the result of this excessive use, large amounts of soil and water resources are in danger of being affected, which is considered a threat to the environment. Atrazine herbicide is extremely toxic to freshwater and rivers organisms, thus this herbicide could be eliminated by biodegradation process[20]. Previous research had reported atrazine biodegradation by bacterial strains such as *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Aerobacterium* family, *Microbacterium*, *Bacillus*, *Micrococcus*, and *Delftia acidovorans*, also by species consortia such as *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Pseudomonas putida*, *Rhizobium* species, and *Nocardia* species[21].

In the present study, two Gram-positive and six Gram-negative bacteria were isolated in the summer, and only five Gram-negative bacteria were isolated in the autumn, all capable of degrading atrazine. In 2011, Rezaei *et al*. studied atrazine degradation by *Pseudomonas fluorescence* and *Pseudomonas aeruginosa* species in vitro. Atrazine was degraded by both *Pseudomonas* species at the temperatures of 32° C for 48 hours, and the degradation ability of *Pseudomonas fluorescence* was more than *Pseudomonas aeruginosa*[7]. In 2012, Shahitha isolated bacteria capable of consuming atrazine, from soil and water samples collected around the sugarcane farms. According to chemical tests, the isolates identified as *Bacillus* sp., *Alcaligenes* sp. and *Pseudomonas* sp. that all isolated bacteria in previous research were able to degrade atrazine, similar to present study. However, unlike current research, the strain of *Pseudomonas* was isolated as atrazine-degrading bacteria in most of previous research[22].

Rivers are in direct contact with the atmosphere. Thus, the intensity and duration of sun exposure could increase, especially in the warm seasons. Indeed in most cases, pesticide degradation occurs faster in warm and sunny days[23]. Many factors contribute the length of time herbicides persist including soil factors, climatic conditions, and herbicidal properties. These factors strongly interact with one another. Some herbicides including atrazine are particularly affected by soil pH, which is an important part of the soil chemical makeup[24]. Temperature plays an important role because it reduces the rate of chemical reactions and biotic activity[25].

In terms of weather conditions, summer is a warm season with high diversity of bacterial strains. The Onset of autumn, beginning of rainfall and falling temperature causes more restrictive conditions for the growth of various bacteria. This fact supports the finding that the counted bacteria in summer was estimated 4.965±0.016 cfu/ml, larger compared with 4.894±0.017 cfu/ml in autumn. The results also showed that the abundance of Gram-negative bacteria was larger than gram-positive bacteria in the summer (77% compared with 23%), whereas no Gram-positive bacterium was found in the autumn. Based on high percentage of Gram-negative bacteria in both seasons, it is probable that Gram-negative bacteria are more resistant because of more complicated wall structure. Changing in climate could cause some changes in the frequency and diversity of degrading bacteria in different seasons. The findings of this research showed that atrazine-degrading bacteria are significantly distributed in the Karun River sediments, and isolated atrazine-degrading bacteria

are predominantly indigenous species, having the ability to decompose compounds.

In 2012, Umar *et al*. suggested that moisture and temperature could be affected as changes occur through seasonal changes[26].

In 2007, Hager and Nordby studied herbicide persistence in soil and stated that the climatic variables involved in herbicide degradation are temperature, moisture, and sunlight. Herbicide degradation rates generally raise with increased temperature and moisture because of increase in both chemical and microbial decomposition rates. Cool and dry conditions slow degradation and consequently cause greater potential stability. If the seasonal conditions are mild and wet, herbicide persistence is less likely. Based on these reasons, degradation will accelerated on sunny days[27].

In 2008, Przybulewska and Sienika studied decomposition of atrazine by microorganisms isolated from long-term herbicide experiment soil. Some microorganisms were reported to have abilities to degrade xenobiotic, using them as a source of carbon, nitrogen, and energy. Biodegradation has been reported to be influenced by many factors such as temperature, pH and most importantly the composition and the activity of soil microorganism's community and the degradability of compounds[28].

The present research findings confirm the effect of previous mentioned factors including temperature on the quantity and frequency of identified atrazine-degrading bacteria in two focused season, in Karun River. Based on the fact that the absolute temperature is higher in summers, the temperature of water resources increases. Consequently, diversity and quantity of bacteria resistant to atrazine would be higher, and these all result in more atrazine biodegradation in summers compared with autumns. In summary, the seasonal temperature change is an effective factor on the number of bacteria. Final results also show all of the isolated degrading bacteria are able to degrade atrazine, and they could be effective selections for atrazine bioremediation in the environment.

CONCLUSION

To summarize, the findings of this study showed that each station contained different number of bacteria. Atrazine herbicide degrading bacteria were proven to have a broad distribution in Karun River sediments, and these kinds of isolated atrazine-degrading species were mainly indigenous bacteria with the potential ability to degrade this compound. A significant difference was found at p-value of 1% and 5% between the seasonal amounts of bacteria, Gram-positive and negative bacteria, the sampling stations, and the groups with presence and absence of atrazine.

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REFERENCES

1. Vargha, M. Genetic and ecophysiological survey of atrazine degradation soil and sediment bacteria. [Dissertation]. Hungary: Suzet Istvan Univ., 2002.
2. Radosevich, M., Traina, S.J., Yue, L., Tuovinen, O.H. Degradation and mineralization of atrazine by a soil bacterial isolate. *Appl. Environ. Microbiol*., 1995; **61**(1): 297-302.
3. Hernandez, M., Morgante, V., Flores, C., Villalobos, C., Gonzalez, M., Miralles, P., Dinamarca, A., Seeger. M. Modern approaches for the study of s-triazine herbicide bioremediation in agricultural soils. *J. Soil Sc. Plant Nutr*., 2008; **8**(2): 19-30.
4. Ghosh, PK., Philip, L.. Environmental significance of atrazine in aqueous systems and its removal by biological processes, an overview. *Global Nest J*., 2006; **8**(2): 159-178.
5. Solomon, R.D.J., Kumar, A., Santhi, V.S. Atrazine biodegradation efficiency, metabolite detection and trzd gene expression by enrichment bacterial cultures from agricultural soil. *J. Zhejiang Univ. Sci. B*., 2013; **14**(12): 1162-1172.
6. Iker, B.C., Kambesis, P., Oehrle, S.A., Groves, C., Barton, H.A. Microbial atrazine breakdown in a karst groundwater system and its effect on ecosystem energetic. *J. Environ. Qual*., 2010; **39**(2): 509-518.
7. Rezaei, D., Haghnia, Gh., Lakzyan, E., Khayyat, M.H., Nasirly, H. Study of atrazine biodegradation by Pseudomonas fluorescence and *Pseudomonas Aeruginosa* (In Vitro). *Journal of Water and Soil (Agricultural Sciences and Technology)*., 2011; **25**: 799-806.
8. Bi, H.T., Zhang, L.Y., Liy, N., Zhu, BI.

Preparation of bio-beads and their atrazine degradation characteristics. *Chem. Res. Chinese Unversities*., 2011; **27**(3): 407- 412.

9. Struthers, J.K., Jayachandran, K., Moorman, T.B. Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. *Appl. Inviron. Microbio*l., 1998; **64**(9): 3368-75.
10. Radosevich, M., Tuovinen, O.H. Microbial degradation of atrazine soils, sediments and surface water. *American Chemical Society*., 2002; **42**(2): 432-436.
11. Zablotowicz, R.M., Weaver, MA., Locke, M.A. Microbial adaptation for accelerated atrazine mineralization/degradation in Mississippi Delta soils. *Weed Sci*., 2006; **54**: 538-547.
12. Siripattanakul, S., Wirojanagud, W., McEvoy, J., Limpiyakorn, T., Khan, E. Atrazine degradation by stable mixed cultures enriched from agricultural soil and their characterization. *J. Appl. Microbiol*., 2009; **106**(3): 986-992.
13. Abigail, EA., Das, N. Microbial degradation of atrazine, commonly used herbicide. *I.J.A.B.R*., 2012; **2**(1): 16-23.
14. Dehghani, M., Nasseri, S., Amin, S., Naddafee, K., Taghavi, M., Yunesian, M., Maleky, N. Isolation and identification of atrazine_degradating bacteria from corn field in Fars province of Iran. *Pak. J. Biol. Sci*., 2007; **10**(1): 84-89.
15. Cai, B., Han, Y., Liu, B., Ren, Y., Jiang, S. Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China. *Lett. Appl. Microbiol*., 2003; **36**(5): 272-276.
16. Hosseini, SA., Mirvaghefi, A., Alishahi, M., Khorasani, N. Measurement of blood indices of common carp caught from contaminated region (Ahvaz) and noncontaminated region (Shushtar), Karun River. The 17th National & 5th International Iranian Biology Confrence, Shahid Bahonar University, Kerman, Iran. 4-6 September. 2012.
17. Udeani, T.K.C., Obroh, A.A., Okwuosa, C.N., Achukwu, P., Azubic, N. Isolation of bacteria from mechanic work shop soil environment contaminated with used engine oil. *Afr. J. Biotechnol*., 2009; **8**(22): 6301-630.
18. Kafilzadeh, F., Hosseini, S.Z., Izadpanah, P., Jamali, H. Isolation and identification of carcinogen acenaphthene-degrading endemic bacteria from crude oil contaminated soils around Abadan refinery. *J. Fasa Univ. Med. Sci*., 2012; **2**(3):181-186.
19. Schaad, N.W., Jones, JB., Chun, W. Laboratory guide for identification of plant pathogenic bacteria. 3rd ed. American Phytopathological Society Press, St. Paul, MN, 2001.
20. Haghnia, GH., Razavi, E. Remediation of soil and water contaminated with pesticides. 1st edition. Environmental Protection Agency, Tehran, Iran. 2008.
21. Sene, L., Converti, A., Secchi, G.A.R, Simao, R.C.G. New aspects on atrazine biodegradation. *Braz. Arch. Biol. Technol*., 2010; **53**(2): 487-496.
22. Shahitha, S. Studies on atrazine mineralization by a consortium of bacteria isolated from sugarcane field soil. *Journal of Applied Pharmaceutical Science*., 2012; **2**(6): 223-226.
23. Kupaei, J.A., Nasiri, Z., Talebi, Kh., Mamunpoush, A., Moosavi, S. Investigation of Zayandehrud water pollution by diazinon and its assimilative capacity. *J. Sci. Technol. Agric. Nat. Resour*., 2011; **15** (56):1-20.
24. Hager, A., Sprague, C., McGlamery, M. Factors Affecting Herbicide Persistence. Illinois agricultural pest management handbook. Illinois: University of Illinois. 2000., Chapter 20: 323-326.
25. Lee, K.E., Huggins, D.G., Thurman, E.M. Effects of hydrophyte community structure on atrazine and alachlor degradation in wetlands. In: Campbell, K.L. (Ed), Versatility of wetlands in the agricultural landscape, American Society of Agricultural Engineers, St. Joseph, MO., 1995; 525-538.
26. Umar, A.F., Tahir, F., Larkin, M., Oyawoye, O.M, Musa, B.L., Yerima, M.B., Agbo, E.B. In-situ biostimulatory effect of selected organic wastes on bacterial atrazine biodegradation. *Advances in Microbiology*., 2012; **2**(4): 587-592.
27. Hager, AG., Nordby, D. Herbicide persistence and how to test for residues in soils. Illinois agricultural pest management handbook. Illinois: University of Illinois. 2007., Chapter 15: 343-350.
28. Przybulewska K and Sienicka K. Decomposition of atrazine by microorganisms isolated from long-term herbicide experiment soil. *Ecol. Chem. Eng. S*., 2008; **15**(4): 492-499.