



Comparison of Some Soil Fungi in Bioremediation of Herbicide Acetochlor Under Agitated Culture Media

Gokhan Onder Erguven¹

Received: 3 October 2017 / Accepted: 17 January 2018 / Published online: 24 January 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

In this study, we aimed to find out the biodegradation efficiency of some soil fungi types on herbicide acetochlor with acetochlor active material, chemical oxygen demand (COD), biochemical oxygen demand (BOD₅) and total organic carbon (TOC) parameters. We also try to determine the population dynamics of these fungi via turbidity. The fungi cultures were isolated from agricultural field in in Thrace region of Turkey. Each fungi enriched in malt extract broth media and 1 mL of these enriched media was inoculated into 100 mL of acetochlor solutions that suggested for using application concentrations in agricultural field for farmers (11000 mg L⁻¹) for sunflower and corn agriculture. Bioremediation results of acetochlor have shown different results according to the types of fungi. Our results indicate that *Tolypocladium geodes* and *Cordyceps cicadae* might be used in acetochlor bioremediation with a significant acetochlor, COD, TOC and BOD₅ reduction and can be used for rehabilitation of agricultural fields or receiving environments for removal the opposite effects of acetochlor or other herbicides.

Keywords Soil fungi · Acetochlor · Chemical oxygen demand · Biochemical oxygen demand · Total organic carbon · Turbidity

Pesticides are used on a large scale for agricultural activities. The opposite effects of pesticides on both human health and environment are a matter of common problem. Thus in agricultural products, the residue levels of pesticides should be controlled comprehensively (Tomlin 2000).

The natural degradation process of pesticides in the environment and elimination of them is called bioremediation. This method has many application methods in contaminated environments such as soil, water, sediments, oceans and lakes (Boopathy 2000). Contamination of the receiving environment with pesticides is a major issue in our day. Pesticides used in agriculture may lead to environmental problems when deteriorating the quality of soil and groundwater. In these cases bioremediation can be a suitable solution for remediating the pollution. Bioremediation is simply the use of microorganisms for cleaning up chemical pollution by reducing the concentrations of chemical compounds and restoring natural conditions (Ahemad et al. 2008). During

the last years, there have been significant activities on the use of fungi to increase availability of biodegradation and bioremediation (Boopathy 2000). These fungi are therefore expected to play an important role in the degradation of pesticides present in the aquatic environment.

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide) is a chloroacetanilide herbicide used for control of most annual grasses and certain broadleaf weeds and yellow nutsedge. Crops include cabbage, citrus, coffee, corn (all types), cotton, green peas, maize, onion, orchards, peanuts, potatoes, rape, soybeans, sugarbeets, sugarcane, sunflower, and vineyards. Acetochlor is applied preemergence, preplant incorporated and is compatible with most other pesticides and fluid fertilizers when used at recommended rates (Thomson 1993).

Bioremediation/biodegradation process is eco-friendly method and one of the best solution method that can finish with degradation or transformation of hazardous environmental materials into harmless or less toxic forms (Shan et al. 2009). In this process, microorganisms can degrade/bioremediate pesticides as a source of carbon and energy or by cometabolise them (Mosa et al. 2016). Maintaining knowledge about the biodegradation capabilities of fungi is

✉ Gokhan Onder Erguven
gokhanondererguven@gmail.com

¹ Department of Environmental Engineering, Faculty of Engineering, Munzur University, Tunceli, Turkey

important, since these microorganisms may produce many enzymes capable of degrading pesticides (Oliveira et al. 2015).

There are several studies on the use of fungi, bacteria, actinomycetes and yeast in a consortium for the bioremediation of pesticides in the soil for the purpose of eliminating their dangerous products (Gaylarde et al. 2005). Most of the microorganisms show the biggest capability of degrading pesticides (Vibber et al. 2007).

The aim of this study is to evaluating the efficiency of some soil fungi to degrade the suggested concentration of acetochlor for wheat and corn farmers under agitated (130 rpm) submerged culture conditions via remediation of acetochlor, chemical oxygen demand (COD), total organic carbon (TOC), biochemical oxygen demand (BOD₅) and reveal the starting time of bioremediation/biodegradation process of these fungi. We have also try to suggest the most suitable fungi type for bioremediate/biodegrade agricultural fields and receiving environments polluted with acetochlor herbicide.

Materials and Methods

Tolypocladium geodes, *Cordyceps cicadae*, *Metacordyceps owariensis*, *Metarhizium cylindrosporae* and *Verticillium chlamydosporium* were used in this study. These fungi strains are currently available in our culture collections. The strains were maintained in petri dishes on malt extract agars (MEAs) at 4°C in refrigerator.

Acetochlor active ingredient was obtained from Sigma-Aldrich (Germany) with a cas number of 34256-82-1. MEA and malt extract broth (MEB) were purchased from Sigma-Aldrich (Turkey) with a lot number of M6409 and 70146.

For preparation of inoculum and submerged culture medium, *T. geodes*, *C. cicadae*, *M. owariensis*, *M. cylindrosporae* and *V. chlamydosporium* were cultured at 25°C on MEA slants in glass tube. After 5 days of incubation, conidial suspensions were prepared and used for the preparation of inoculum. 1 mL of the suspension was transferred into a 100 mL flask containing MEB and agitated on a rotary shaker at 130 rpm for 7 days at 25°C. After incubation, flasks homogenized and these homogenized mycelial cultures were used as inoculum for studies under submerged culture medium. 1 mL homogenized mycelial culture was transferred into 100 mL flasks containing 11,000 mg L⁻¹ of acetochlor (suggested concentration for corn and sunflower farmers) on an agitated incubator for 7 days at 25°C in triplicate. After incubation, all flasks filtered for determinate acetochlor, COD, BOD and TOC reduction rates.

Each part of the samples are used for acetochlor determination, COD, TOC and BOD₅ studies. EPA Method 535 (Measurement of chloroacetanilide and other acetamide herbicide

degradates in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (lc/ms/ms)) used for acetochlor determination with a Dionex Ultimate 3000 model device, with C18 Thermo Accucore columns have a dimension of 2.6 μ, 2.1 mm × 100 mm. The autosampler temperature was 50°C and furnace temperature was 400°C. The retention time was 8.00 min. (Thermo Access Max) The ion transfer tube temperature was 270°C and HESI ion source was 3500 V. While the amount limit was 15 ppb, the Aux and Sheath gases were 15 Arb and 50 Arb, respectively. The collision gas pressure was 1.5 m Torr. The LOD and LOQ values were 4 ppt and 15 ppb. The ion transfers were 285.6 for the primary ion, 194.6 for the secondary ion, and 266.7 for the tertiary ion. The amount limit (method marker) was 0.1–1 mg kg⁻¹. In the calibration curve, the average accuracy value was (R^2) 0.999. In order to maintain the sensitivity, the samples were spiked with internal and surrogate and standards. As the surrogate standard, tetrachloro-mxylene (TMCX) was used while Quintozene was used as the internal standard. The surrogate standard was added to the samples before extraction and chromatograph vials were spiked before they were capped. The average recovery sensitivity was 91%. For each species, the limit of detection (LOD) was calculated/measured by the addition of the average witness value 3 three times of the standard deviation value. Values below the LOD value were not considered in the measurement. Witness samples were verified in each set of analysis, and all of the results were subject to witness verification. Closed titrimetric method was used for the COD experiments identified in the Standard Method 5220C and decreasing of the substrate followed by day. BOD₅ test was conducted with Standard Method 5210B (5 day BOD₅) test and the TOC test was performed in line with the method of burning at a high temperature identified in the Standard Method 5310A with TEKMAR - DOHRMANN - Apollo 9000 device (APHA 1998) Additionally, to reveal the population dynamics of these fungi, turbidity measurements taken from media were performed at 650 nm (Photolab 6600 UV-VIS Spectrophotometer) according to Harry et al. (1990) All of the measurements were performed at room temperature (25°C). All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The value presented are the averages of the results of three replicates of each experiments with a standard error (SE). To compare the acetochlor, COD, BOD₅ reduction, turbidity and colony number (N) in media, the data were analyzed by analysis of variance (ANOVA).

Results and Discussion

Acetochlor, COD, TOC and BOD₅ removal efficiency results obtained from agitated culture media for all fungi types are shown in Figs. 1, 2, 3, 4 and 5 respectively. The population

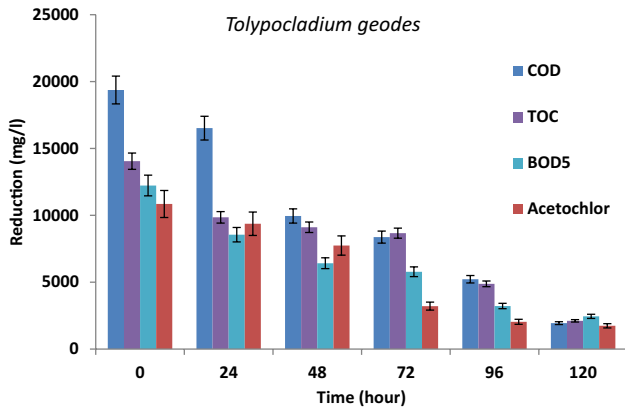


Fig. 1 Reduction of acetochlor by *Tolypocladium geodes*

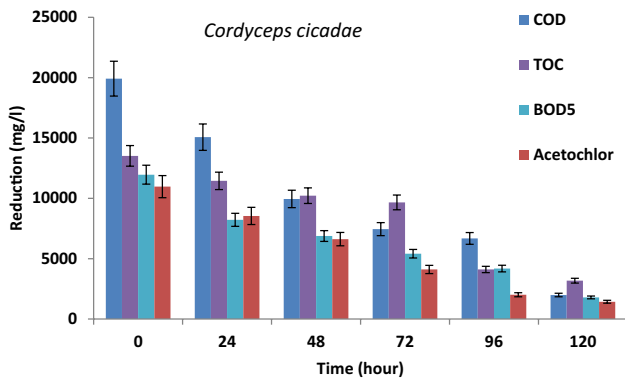


Fig. 2 Reduction of acetochlor by *Cordyceps cicadae*

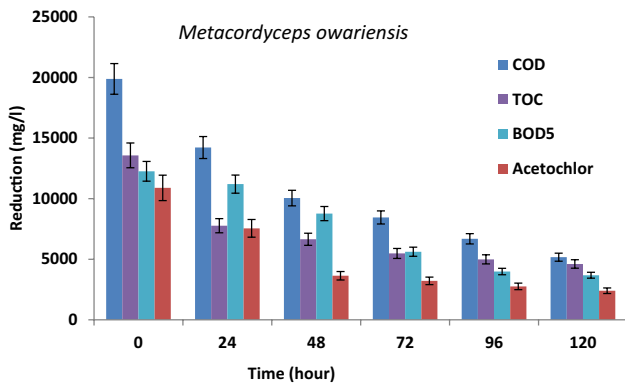


Fig. 3 Reduction of acetochlor by *Metacordyceps owariensis*

dynamics of all fungi are shown in Figs. 6, 7, 8, 9 and 10 respectively.

Different removal efficiency in the submerged culture media have showed different results depend on differences in fungal species. The initial (0th day) acetochlor, COD, TOC and BOD₅ values of the agitated culture solutions were about 11,000, 18,500, 13,000 and 11,000 mg L⁻¹ respectively. Hai

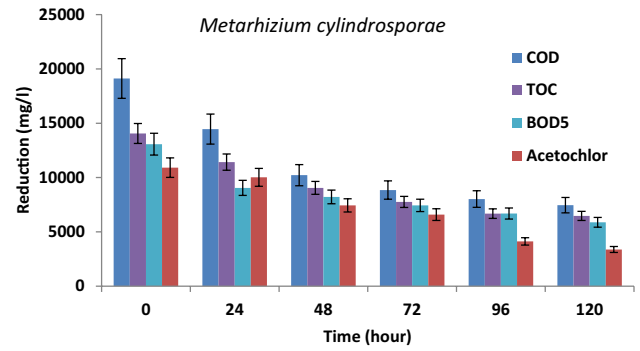


Fig. 4 Reduction of acetochlor by *Metarhizium cylindrospora*

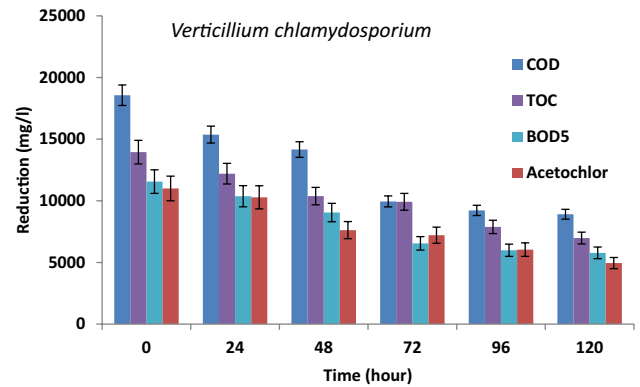


Fig. 5 Reduction of acetochlor by *Verticillium chlamydosporium*

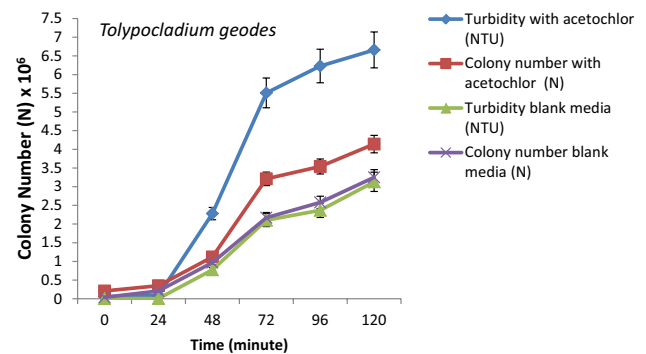


Fig. 6 Population dynamic of *Tolypocladium geodes* related with turbidity

et al. (2012) suggested that the biodegradation of some pesticides was mainly due to activity of fungi, however, pesticides were not degraded when fungi were separately inhibited. The reduction rate of acetochlor by *T. geodes* and *C. cicadae* were 91% and 87% respectively. The COD reduction efficiencies of these two fungi were 90% for COD and 85% for TOC, while *M. owariensis*, *M. cylindrospora* and *V. chlamydosporium* were 78%, 69% and 55% for acetochlor, 74%, 61% and 52% for COD and 70%, 55% and 50% for

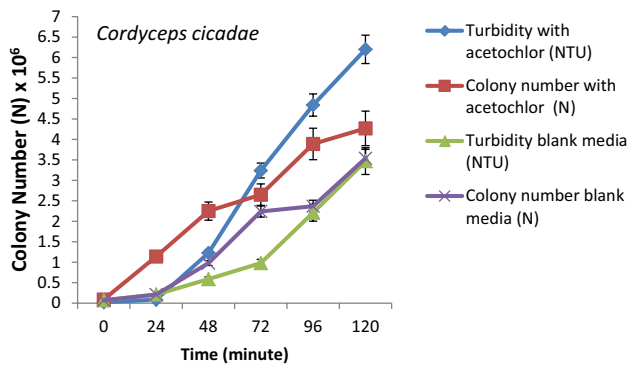


Fig. 7 Population dynamic of *Cordyceps cicadae* related with turbidity

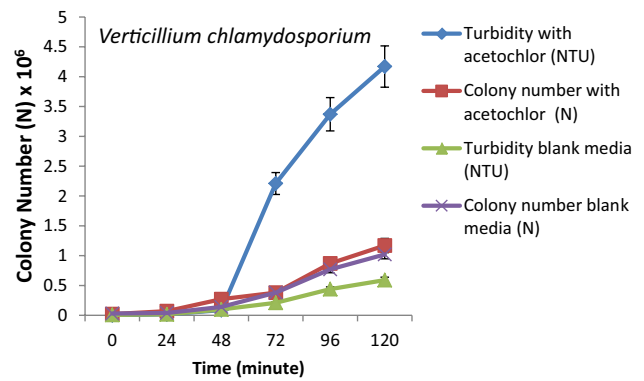


Fig. 10 Population dynamic of *Verticillium chlamyosporium* related with turbidity

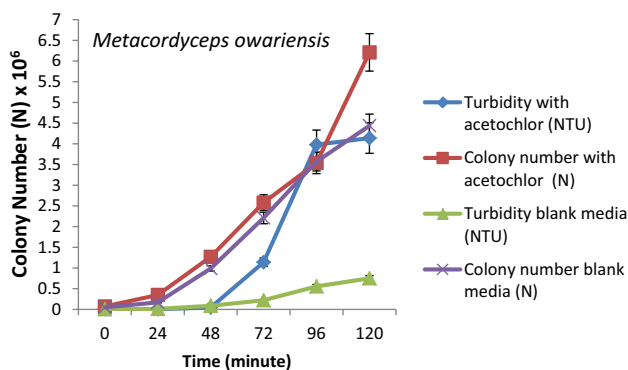


Fig. 8 Population dynamic of *Metacordyceps owariensis* related with turbidity

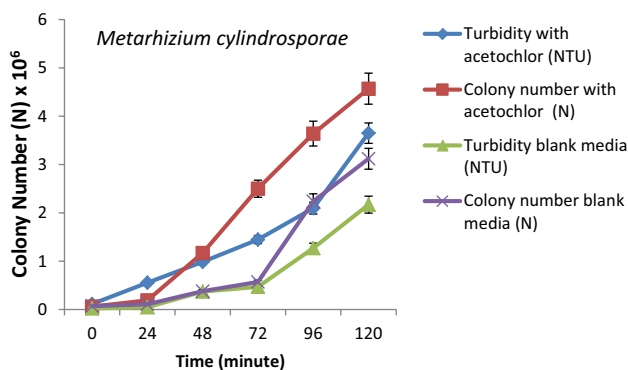


Fig. 9 Population dynamic of *Metarhizium cylindrospora* related with turbidity

TOC respectively in 5 days. The reduction rates for BOD₅ were 80%, 76%, 66%, 54%, and 50%, at the end of the same time. There were negligible changes in acetochlor, COD, TOC and BOD₅ parameter at the end of 6th day for each fungi, so the result of the 6th days were ignored. Previous

studies on degradation of some herbicides via fungi revealed that fungi types were able to degrade herbicides. There have been several studies about removal of pesticides via microbial degradation from soil and water (Awad et al. 2011). Yang et al. (2014) studied a bacterial strain with the ability to utilize chlorimuron-ethyl (herbicide) as the carbon source in Phosphate-basal minimal medium cultures. They found chlorimuronethyl was provided as the sole carbon source, the increase of growth rate of microbial strain accompanied with the degradation of chlorimuronethyl, and more than 95% of chlorimuron-ethyl at an initial concentration of 50 mg L⁻¹ was degraded at the end of a 4th day. Boschini et al. (2003) studied degradation of herbicide chlorsulfuron by *Aspergillus niger*. As a result, after 4 weeks, the chemical degradation excluded, were about 30% for chlorsulfuron. Previous study about biodegradation of the chlorsulfuron herbicide, the COD reduction rates of *Bacillus simplex*, *Bacillus muralis*, *Micrococcus yunnanensis*, *Micrococcus luteus* and *Clostridium tetani* species were 94%, 78%, 79%, 70% and 74%, respectively at the end of the 108th h (Erguven and Yildirim 2016). Erguven et al. (2016) prepared five different units with the soil samples taken from the Thrace Region and added 1900 µg L⁻¹ aclonifen (one of the other herbicide) to each of them. According to the results of their study, the highest bioremediation was observed in the soil sample to which 10 mL of mixed culture of microorganisms was added and Aclonifen, COD, BOD, and TOC remediation was observed as 93.2%, 97.8%, 98.8%, and 98.7%, respectively at the end of the 5th week. The witness sample, which did not include any mixed culture of microorganisms, displayed 49% aclonifen remediation at the end of the same time which suggests that the half-life of the pesticide and the adsorption mechanism were a factor. Among the mixed cultures that were grafted in different concentrations, the highest remediation performance resulting from degradation of microorganisms was 93% in the soil ecosystem that was placed in the 10 mL (approximately 10⁹ CFU/mL) mixed

culture. In the same environment, the COD, BOD, and TOC remediation performances were approximately 98%, 99%, and 99%, respectively, at the end of the 5th week. Mawgoud (2005) investigated the biodegradation potential of *Pseudomonas putida* and found that this type of bacterium has a significant capacity for insecticide malathion degradation. According to the results, degradation rate of insecticide was occurred at a concentration of 125 mg L⁻¹ about 72%. Belal and Mohamed (2013) performed a study on bioremediation of herbicide pendimethalin with isolated *P. putida* bacteria from pendimethalin polluted soil and at the end of 4 weeks; they observed that all 100 µg/mL concentration of pendimethalin was removed by that bacteria species. This means that, remediation process at the soil ecosystem (pot studies) continued about 5 weeks while in agitated culture media, continued about 5 or 6 days. Yonten et al. (2017) tried to reveal the biodegradation performance of *P. eryngii* var. *ferulae* for COD parameter and they found *P. eryngii* var. *ferulae* was a suitable fungi type for bioremediation of pesticides. Experimental results on monitoring fungal activity in the medium with acetochlor showed a slight increase in turbidity, particularly after from the 24th min on *T. geodes* and *C. cicadae* while *M. owariensis*, *M. cylindrospora* and *V. chlamydosporium* showed this increase after the end of the 48th min in media with or without acetochlor. This means that these times are for adaptation of the fungi for bioremediating acetochlor and can be same or different for all types of fungi.

The data reported in this study indicate that microorganisms can survive by degrading a herbicides. Most fungi inhabit agricultural soil, enabling them to degrade pesticides more rapidly. Furthermore, since using chemical and physical methods to degrade pesticides is very expensive and difficult, several researchers suggested the use of these cultures for this purpose. In recent years, several projects and studies on bioremediation of agricultural fields from herbicides were conducted. In this study, we found that *T. geodes* and *C. cicadae* fungi might be used in bioremediation practices in soil polluted by acetochlor. These fungi also can be use for removal pesticide pollution from agricultural fields and receiving environments. Acetochlor, COD, TOC and BOD₅ parameters can give us a valuable information for understanding the removal efficiency of pesticides.

In this study, it was observed that removal rates of acetochlor in agitated culture media obtained by *T. geodes*, *C. cicadae*, *M. owariensis*, *M. cylindrospora* and *V. chlamydosporium* were 91%, 87%, 78%, 69% and 55% as acetochlor, 90%, 90%, 74%, 61%, and 52% as COD; 85%, 85%, 70%, 55% and 50% as TOC, finally 80%, 76%, 76%, 54% and 50% as BOD₅ respectively. According on these reduction rates, it could be concluded that *T. geodes* and *C. cicadae* have the highest removal rate for each parameters. As a result of this study, it was observed that *V. chlamydosporium* had the

lowest acetochlor, COD, TOC and BOD₅ removal efficiency. We sure that, there was a suitable fungi species in agricultural fields for bioremediation of herbicide contaminated liquid media. For further insight into the results, the kinetics of pesticide removal and its toxic effect on fungi cultures, bioremediation performance of these cultures in pot studies need to be studied.

Acknowledgements Thanks to Assist. Prof. Dr. Hurrem Bayhan for his valuable comments for graphics and results.

References

- Ahemad M, Khan MS, Zaidi A, Wani PA (2008) Remediation of herbicides contaminated soil using microbes. In: Khan MS, Zaidi A, Mussarrat J (eds) *Microbes in sustainable agriculture*. Nova Science Publishers Inc., New York, pp 261–284
- APHA (1998) Standard methods for the examination of water and wastewater. 20th edn. American Public Health Association, Washington, DC
- Awad N, Sabit HH, Abo-Aba SEM, Bayoumi RA (2011) Isolation, characterization and fingerprinting of some chlorpyrifos-degrading bacterial strains isolated from egyptian pesticides polluted soils. *Afr J Microbiol Res* 5(18):2855–2862
- Belal BE, Mohamed FEN (2013) Bioremediation of pendimethalin contaminated soil. *Afr J Microbiol Res* 7(21):2574–2588
- Boopathy R (2000) Factors limiting bioremediation technologies. *Bioresour Technol* 74(1):63–67
- Boschin G, D'Agostina A, Arnoldi A, Marotta E, Zanardini E, Negri M, Valle A, Sorlini C (2003) Biodegradation of chlorsulfuron and metsulfuron-methyl by *Aspergillus niger* in laboratory conditions. *J Environ Sci Health Part B* 38(6):737–746
- Erguven GO, Yildirim N (2016) Efficiency of some soil bacteria for chemical oxygen demand reduction of synthetic chlorsulfuron solutions under agitated culture conditions. *Cell Mol Biol* 62(6):92–96
- Erguven GO, Bayhan H, Demir G, Ikizoglu B, Kanat G (2016) Monitoring aconifen remediation in soil with a laboratory scale research. *J Chem Article ID* 5059049:1–8
- Gaylarde CC, Bellinaso ML, Manfio GP (2005) Technical and biological aspects of bioremediation of xenobiotics. *Biotechnol Sci Dev* 34:36–43
- Hai FI, Modin O, Yamamoto K, Fukushi K, Nakajima F, Nghiem LD (2012) Pesticide removal by a mixed culture of bacteria and white-rot fungi. *J Taiwan Inst Chem Eng* 43(3):59–62
- Harry WS, Paul JV, John JLE (1990) *Microbes in action: a laboratory manual of microbiology*, 4th edn. W. H. Freeman, New York
- Mawgoud YA (2005) Molecular characterization of malathion biodegrading enzymes extracted from Egyptian bacterial isolates. *New Egypt J Microbiol* 10:226–231
- Mosa KA, Saadoun I, Kumar K, Helmy M, Dhankher OP (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids. *Front Plant Sci* 7:303
- Oliveira BR, Penetra A, Cardoso VV, Benoliel MJ, Barreto Crespo MT, Samson RA, Pereira VJ (2015) Biodegradation of pesticides using fungi species found in the aquatic environment. *Environ Sci Pollut Res* 22:11781–11791
- Shan XIE, Junxin LIU, Lin LI, Chuanling QIAO. (2009) Biodegradation of malathion by *Acinetobacter johnsonii* MA19 and optimization of catabolism substrates. *J Environ Sci* 21(1):76–82
- Thomson WT (1993) *Agricultural chemicals. Book II: herbicides*. Thomson Publications, Fresno

- Tomlin C (ed) (2000) The pesticide manual, 12th edn. British Crop Protection Council and Royal Society of Chemistry, London
- Vibber LL, Pressier MJ, Colores GM (2007) Isolation and characterization of novel atrazine-degrading microorganism from an agricultural soil. *Appl Microbiol Biotechnol* 75:921–928
- Yang L, Li X, Li X, Sua Z, Zhanga C, Zhang H (2014) Bioremediation of chlorimuron-ethylcontaminated soil by *Hansschlegelia* sp. strain CHL1 and the changes of indigenous microbial population and N-cycling function genes during the bioremediation process. *J Hazard Mater* 274:314–321
- Yonten V, Alp H, Yildirim N, Yildirim NC, Ogedey A (2017) Investigation of optimum conditions for efficient COD reduction in synthetic sulfamethazine solutions by *Pleurotus eryngii* var. *ferulae* using response surface methodology. *J Taiwan Inst Chem Eng* 80:349–355