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Can Toxicities Induced by Insecticide Methomyl be Remediated Via Soil Bacteria *Ochrobactrum thiophenivorans* and *Sphingomonas melonis?*

Sule Tatar[1] · Nuran Cikcikoglu Yildirim[2] · Osman Serdar[3] · Gokhan Onder Erguven[1]

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Abstract
The research study was about revealing the biochemical response of *Gammarus pulex* related to insecticide methomyl before and after bioremediation by two soil bacteria species, *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*. Catalase (CAT), glutathione *S*-transferase.(GST), cytochrome. P4501A1 (CYP1A1) activities in *G. Pulex* related to methomyl solution were investigated in 24 h and 96 h. ELISA method was used for test studies. CAT enzyme was decreased in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P < 0.05$). CAT activities were returned to control results after bioremediation assays. GST enzyme activity was decreased depending on methomyl exposure during 24 h but increased during 4 days ($P < 0.05$). After 8 days of bioremediation period, GST activity increased again during 24 h while decreased during 4 days ($P < 0.05$). CYP1A1 activity increased in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P > 0.05$). After bioremediation, statistically significant changes were not revealed in CYP1A1 activities ($P > 0.05$). According to the results of our study, CYP1A1, CAT, and GST activities in *G. pulex* sanctioned the capability of *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* in methomyl bioremediation. Isolated and enriched *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* that were added to 2.5 ppb concentrations of methomyl for 8 days. Each day, chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5), pH and dissolved oxygen parameters were monitored. At the final phase of the bioremediation step, it was determined that these bacteria have efficient methomyl bioremediation properties in a mixed corsortia at a rate of 86%. These results show that these bacteria can be used for bioremediate the receiving environments that are polluted by these kinds of insecticides.

Introduction

Methomy [*S*-methyl *N*-((methylcarbamoyl)oxy) thioacetimidate] is generally used for handle too many insects kinds, on a wide range of agricultural crops. Methomyl, that functions as an AChE-inhibiting systemic insecticide, has been known as a highly toxic for aquatic habitat [1]. This insecticide has the properties to convince oxidative damage as evidenced with increase of lipid peroxidation in various antioxidant enzymes [2]. Insecticides indicated to cause over-production of reactive oxygen species (ROS) in extracellular and intra spaces of the cells thereby inducing oxidative stress [3]. Various xenobiotics cause oxidative stress from the production of ROS [4]. To avoid oxidative stress, cells activate detoxification processes through antioxidants in order to reduce toxic effects. CAT has a significant profile in the cellular antioxidant defense to an excessive formation of ROS. CAT is widely used biomarker of oxidative stress and also converts hydrogen peroxide to water and oxygen [5]. This means, CAT involves in the biotransformation process with the accumulation of ROS indirectly [6]. GST serves a main role in the biotransformation of too many components and with a wide variety of organic compounds, catalyze the conjugation of GSH [7]. Cytochrome p450 enzymes are responsible for most biotransformation steps of, xenobiotics and endogenous molecules [8]. Oxidative damage related to methomyl regards bioactivation by the microbial cytochrome P450-dependent monooxygenase system, resulting in the development of sulfoxide, oxoninitiate, and sufone that commence protein oxidation and lipid peroxidation [9]. Among

✉ Gokhan Onder Erguven
gokhanondererguven@gmail.com

[1] Department of Chemistry and Chemical Processes, Tunceli Vocation School, Munzur University, 62000 Tunceli, Turkey

[2] Department of Veterinary Medicine, Laboratorian and Veterinarian Health Programme, Pertek Sakine Genc Vocation School, Munzur University, 62000 Tunceli, Turkey

[3] Faculty of Fisheries, Munzur University, 62000 Tunceli, Turkey

the invertebrates, amphipods of the genus Gammarus, and particularly *Gammarus pulex* (Crustacea, Amphipoda), are widely used for the assessment of toxic effects of pollutants [10].

In the present study, we examine the biochemical response of *Gammarus pulex* exhibited to methomyl insecticide bioremediated by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*. The modification of GST, CYP1A1, and CAT enzymes in *G.pulex* was tested before and after the remediation.

Materials and Methods

Chemicals

Methomyl was obtained from Merck, Turkey, with a CAS number of 16752-77-5. The experimental kits used for biochemical assays of cytochrome P4501A1 (CYP1A1) were obtained from the Cusabio company (catalog no: CSB-EL006395FI), while GST and CAT assay kits were purchased from the Cayman Chemical company with a catalog numbers, 703302 and 707002, respectively. COD kites were obtained from Hach Lange, Turkey, with a product code of LCK114.

Animals

Gammarus pulex were collected from Munzur River (39.156822 N, 39.499642 E) (Fig. 1). They were quickly taken into plastic steriline bottles and taken to the laboratory at 18 °C, a 12:12-light and dark cycle and fed willow leaves for 2 weeks before experimental studies started. For the study, samples in similar intermoult stage measured about 1 cm length using a binocular [11]. Each aquarium consists 10 separates with 3 replication and capacity of aquariums was 1000 ml. During the experiments, there is no feeding process for living organisms. The living organisms were checked day by day and non-living organisms were calculated and taken from aquarium. Inactivity was admitted the criterion for death.

Identification of Bacteria

Approximately 10 g of soil sample was taken from a depth of 20 cm before insecticide application and placed in sterile glass jars [12]. The soil diluted to 10^{-4} in 0.8% sodium chlorate isotonic water; and 0.1 ml of this diluted sample was taken and sown into plate count agar and waited for bacterial growth phase completed at 28 °C about 120 h. Growing bacteria in petri dishes were marked as P1 and P2. For identification of bacteria studies, Phire Hot Start II DNA polymerase was used. PCR bands of various lengths (1000–3000 bp) were obtained through bacterial 16S ribosomal general primers. 16S rRNA forward primers were as "AGA GTT TGA TCC TGG CTC AG," while 16S rRNA reverse were as "ACG GCT ACC TTG TTA CGA CTT."

Experimental Design

Biochemical Measurements

The organisms weighed and homogenized by adding salt solution buffered with phosphate at a rate of 1/5 w/v and using homogenizer for enzyme analyses step with ice. The samples homogenized before have been centrifuged for 15 min at 17.000 rpm in a refrigerated centrifuge; supernatants were moved to deep freeze at − 70 °C immediately and kept there until their assessments finished.

Commercial ELISA kits of The Cusabio company were used for determination of CYP1A1 on ELISA reader. The results determined and analyzed according to the manufacturer's instruction.

The activities of CAT and GST were determined by ELISA reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer). Four experimental groups were designed for GST, CYP1A1, and CAT as follows:

A. Control group: Distilled water
B. Blank media: 2.5 ppb 500 ml of methomyl (includes no bacteria, so there is no bioremediation activity)
C. 2.5 ppb 500 ml of methomyl and consortia of *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* (includes approximately 2×10^9 Colony forming unit (CFU)/ml), beginning of the bioremediation phase, 0th day
D. 2.5 ppb 500 ml of methomyl and consortia of *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* (2 ml), 8th day of bioremediation, final phase.

The individuals of *G. pulex* were exposed to these groups for one and four days (*n*: 10 for each group).

Removal of Methomyl via Bioremediation

Firstly, methomyl was prepared in 100-ml Erlenmeyer Flask as 2.5 ppb. After that, enriched 2 ml *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* (each of them contains 1×10^9 Colony Forming Unit) bacteria added to these flasks. In bioremediation step, COD and BOD_5 studies were performed on the liquid samples and the decreasing of the insecticide was monitored in 8 days for every 24-h intervals. The references and devices used for these experiments are given in Table 1.

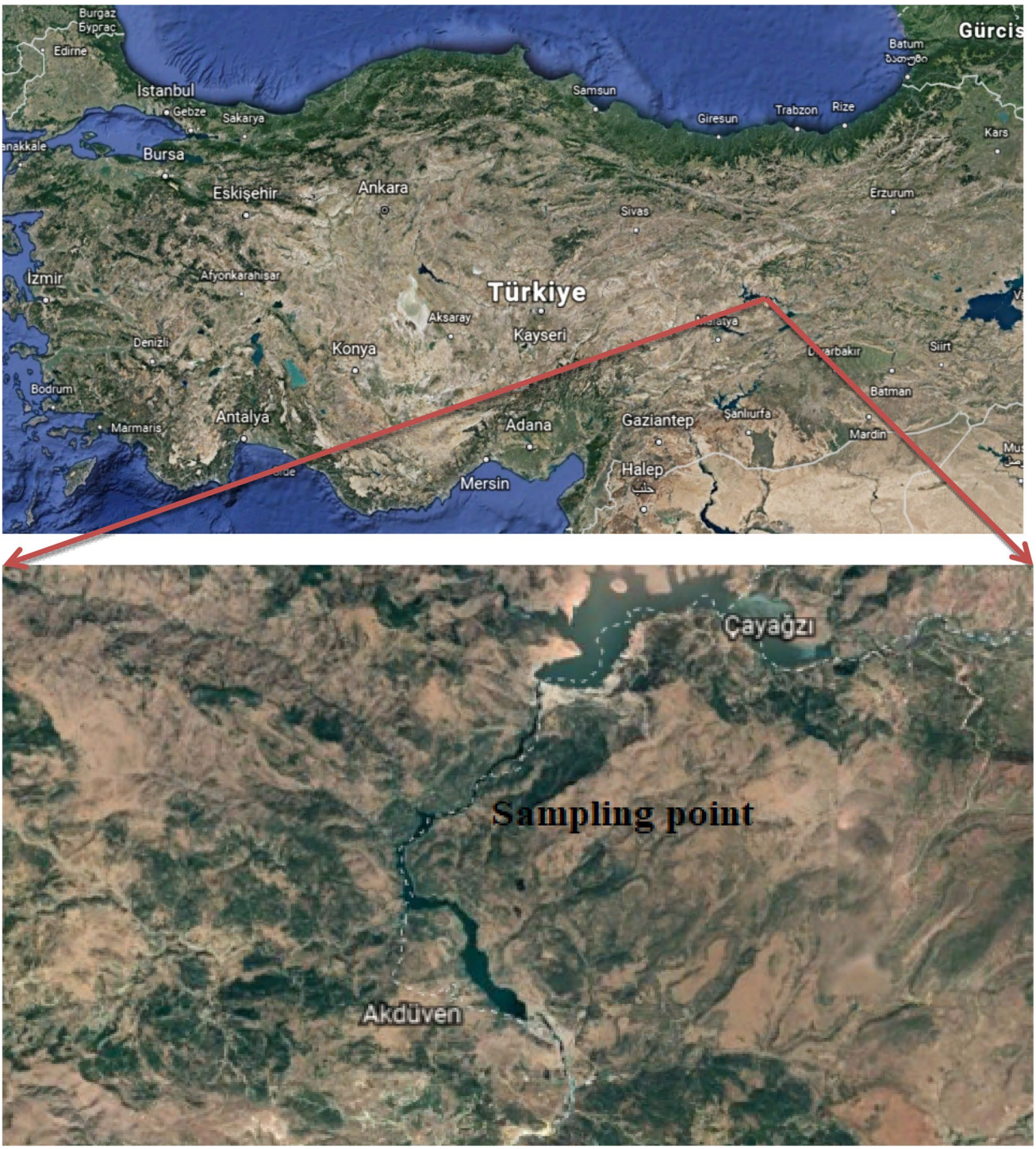

Fig. 1 Sampling point

Table 1 Experiments, devices, and references

Experiment	Device	References
COD	HACH DRB 200 thermoreactor using Hach COD kits that measured in the range 0–1500 mg/l (Cat number: 23459-52)	Standard method 5220C [13]
BOD_5	AL606 Oxitop device	Standard method 5210B (5-day BOD5 test [13]

Biochemical Assays

Prior to biochemical analysis, tissues were homogenized and weighed by adding 1/5 PBS buffer. After homogenization process was completed, it was centrifuged at 17.000 rpm for 15 min. The supernatants obtained were keep at − 70 °C deep freeze until the analysis was performed.

CYP1A1 enzyme activity was determined in microplate reader using commercial kits purchased by cusabio company (Catalog no: CSB-EL006395FI).

Statistical Analyses

The data were analyzed using PASW Statistics 18.0 (SPSSInc., Chicago, IL, USA). One-way ANOVA and the Duncan's multiple range tests were used to evaluate the statistical differences among groups (A, B, C, D, and control group in the same exposure time ($^{abc}P < 0.05$)). Two-tailed independent *t* test was used to compare the differences between the exposure times (1 day and 4 days) in the same group($^{*}P < 0.05$).

Results

The Identification of Species

According to the BLAST software, bacterial species were identified with accession numbers for *Ochrobactrum thiophenivorans (Ot)* as AM490617 while *Sphingomonas melonis (Sm)* as AVM11_16345 with an identity rate of 91% and 90%, respectively, for P1 and P2.

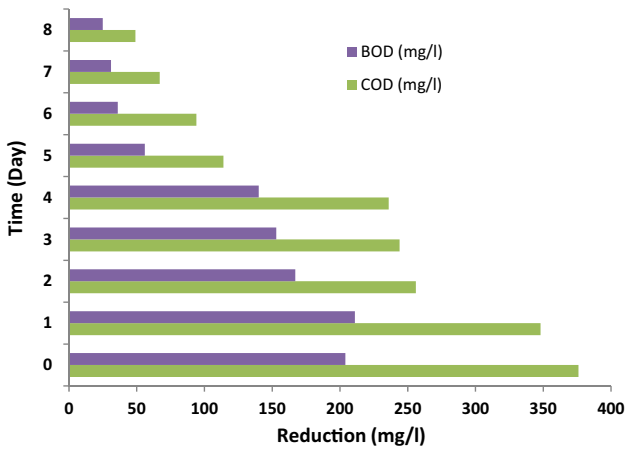

Fig. 2 Reduction of COD and BOD_5

The Bioremediation Results

Since the pesticide was approximately at the beginning COD value in 100 ml mixed media, it was ensured that the bacteria would make its bioremediation process in media with methomyl. Totally 2 ml enhanced *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* bacteria were added to 2.5 ppb 100 ml methomyl solution. The COD and BOD_5 reductions of the liquid media are shown in Fig. 2.

When eight day finished, COD and BOD_5 experiment results showed that consortia of *Ot* and S*m species* showed reduction rates 94.7% and 96.8% (Fig. 1).

Biochemical Parameters

Biochemical parameters of *Gammarus pulex* exposed to methomyl insecticide before and after bioremediation by *Ot* and *Sm* are shown in Figs. 3, 4 and 5.

*Statistical differences according to two-tailed independent *T* test between different exposure time (1 day, 4 days) in same groups $^{*}P < 0.05$, and different letters on bar (a, b, c) show statistical differences of Duncan's multiple range test

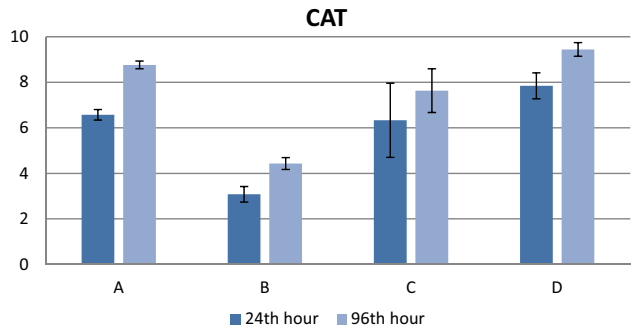

Fig. 3 Antioxidant enzyme response of *Gammarus pulex* related to to methomyl before and after bioremediation by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*

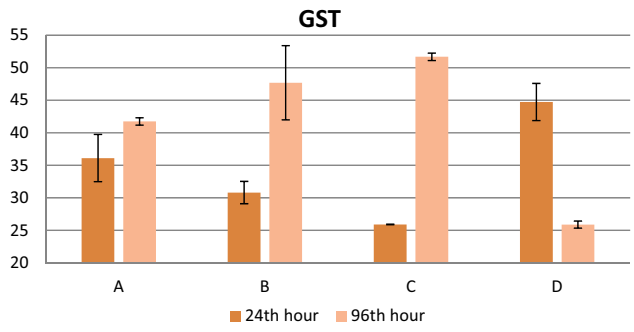

Fig. 4 Detoxification enzyme response of *Gammarus pulex* exposed to methomyl before and after bioremediation by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*

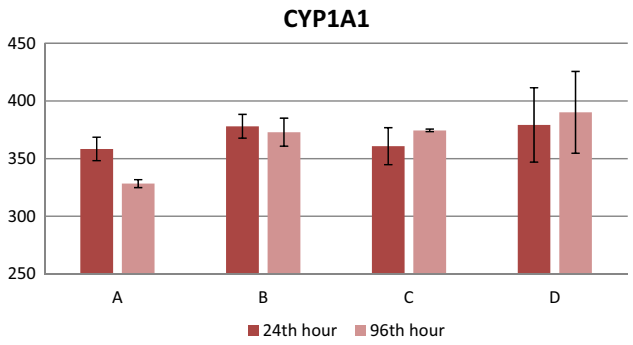

Fig. 5 CYP1A1 enzyme activity of G. pulex exposed to methomyl insecticide before and after bioremediation by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*

among all application groups in the same exposure time, $^{abc}P < 0.05$. Values represent mean ± SE, $n = 10$.

Enzyme activity of CAT was decreased in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P < 0.05$). Activities were turned into control values after bioremediation by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis.*

GST enzyme activity was decreased depending on methomyl exposure during 24 h but increased during 96 h ($P < 0.05$). After 8 days of bioremediation with *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*, GST activity increased again during 24 h but reduced during 96 h ($P < 0.05$).

CYP1A1 activity was increased in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P > 0.05$). After bioremediation, no statistically significant changes were identified in activities for CYP1A1 ($P > 0.05$).

Discussion

Pesticide residuals affected by soil water content and application period of them [14]. The biodegradation of pesticides depends on the microorganisms activities [15]. Erguven and Yildirim (2019) studied Imidacloprid remediation with strains of *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae*. After 18 days, remediation was determined for COD parameter as 52, 96, and 99% with 20, 40, and 80 ml of the consortia of bacteria, respectively, while BOD_5 removal rates were 88, 79, and 50% in the same volumes of bacteria [16]. Most of the researchers thought that some microorganisms have tolerance to the pesticide. In another research study, *B. cereus, B. subtilis, B. melitensis, K. species, P. aeroginosa, P. fluorescence, and S. marcescens* were capable of degrading 46–72% of chlorpyrifos as a sole carbon source in a sedimentary medium after three weeks of incubation period [17].

This means bioremediation can be an efficient and alternative method to remediating environment pollution with methomyl insecticide. Bioremediation of different strains of bacteria was positively enhanced in receiving environments. This means that there was a suitable microorganisms to reduce the opposite effects of insecticides in agricultural areas. To gain knowledge for remediating the fields from the toxic effects of pesticides, it is advised to use microorganism consortia to the contaminated areas after the pesticides gives their opposite effect. BOD_5 and COD are commonly used environmental parameters providing us significant ideas about microbial decomposition of insecticides. Most of the studies related to this study are about using bacteria isolated from active sludges of pesticide producers. But the different perspective of this research study is that the microorganisms were isolated from agricultural field and laboratory scale studies tried to adapt them to methomyl insecticide in first step. Increased bioremediation rate of phenol by immobilized Candida tropicalis on persistent organic pollutants was also demonstrated [18]. The bacterial strains are able in bioreactors because they remain active for up to bioremediation steps [19].

In this study, the consortia of *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae* isolated from Adana province could break down the insecticide with a high removal rate and also could minimize the opposite effects of this material.

The detoxification system protects aquatic species from endo and esogenous aggression and deterioration of this function causes damage of cells. GST are metabolizing phase II enzymes which are involved in the biotransformation of both endogenous substances and xenobiotics [20]. Increased expression in GST and cytochrome P450 have been found in *T. urticae*, European red mite *Panonychus ulmi*, the scabies mite *Sarcoptes scabiei*, hard ticks *Rhipicephalus bursa*, and the *Phytoseiid Phytoseiulus persimilis* that are resistant to many kinds of persistent organic pollutants [21]. Many studies investigating GST enzyme activities in aquatic organisms related to different insecticides showed an enzymatic induction [22]. Mansour et al. (2009) found out that GST activity was reduced by different concentrations of methomyl contaminated rats [23]. GST activity was slightly inhibited in the methomyl exposed *Cyprinus carpio* [24]. Serdar et al. (2019) found that statistically significantly reduced GST enzyme activity depending on dimethoate pesticide in *Gammarus pulex* [25]. El-Demerdash et al. (2012) suggested that methomyl treatment caused a significant decrease in GST [26]. In present study, GST enzyme activity was increased depending on L 90 exposure during 96 h ($P < 0.05$). After 8 days of bioremediation with *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*, GST activity increased again during 24 h but decreased during 96 h ($P < 0.05$). This results is generally expected once the

GST plays a significant role on detoxification of electrophilic compounds.

The effect of the methomyl on mixed function oxidase was studied using different durations, concentrations, and sex. Methomyl treatment of old male rat and adult female rat showed a decrease in the level of cytochrome P450 [27]. In this research study, CYP1A1 activity was spreaded in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P > 0.05$). The CYP1A1 induction and the inhibition of GST activity by methomyl may donate to the toxic effects in *G.pulex.*

CYP1A1 is an interesting biomarker that is sensitive to different concentrations of environmental pollutants and is also related to oxidative stress [28]. Organophosphorus and organochlorine many pesticides have been found to discourage CYP1A1 activity and change the expression [29]. The effect of the methomyl on CYP450 was investigated using different dosages and durations and sex and cytochrome p450 levels have been found to be decreased in adult female rat and old male rat depending on 4 mg/kg methomyl treatment [27]. In the present study, CYP1A1 activity was increased in *Gammarus pulex* that was exposed to methomyl after all exposure period ($P > 0.05$). The CYP1A1 induction and the inhibition of GST activity by methomyl may contribute to the toxic effects of this insecticide in *G. pulex.*

Heikal et al. (2014) indicated that methomyl induced oxidative stress and testicular injures via decreasing the activities of CAT, SOD, and GST enzymes in Wister rat [30]. Similarly, according to our results related of *G. pulex* to methomyl for 24 and 96 h resulted in an important decrease in CAT activity. They also indicated that the activity of CAT was inhibited after 30 days of exposure. When oxidative stress occurs, the defense system against ROS becomes inadequate, and increased level of ROS weakens the antioxidant defense system and reduces intracellular GSH and reduces CAT activity. Hernández-Moreno et al. (2014) studied the effects of the methomyl on different enzymatic activities in *Cyprinus carpio* L. No changes were observed in CAT activity of controlled animals [24]. The CAT enzyme activity is inhibited by the organisms depending on dimethoate exposure [25]. The currently inhibited effects of L90 on CAT in accordance with several studies that report significant changes in CAT activity exposed to different pollutants [31]. In this study, CAT activity was decreased depending on methomyl exposure after bioremediation of the enzyme activity increased again. The decrease in CAT activity can be mainly related to the oxidative stress caused by exposure to methomyl.

According to the results of the study, methomyl could be effectively remediated by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* so this means this bioremediation has a major role in oxidative stress status of *G. pulex*. CAT and GST activities of *G. pulex* are useful biomarkers for determining the efficiency of methomyl remediation with *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*. It has been found that methomyl stimulates oxidative stress. Exposure time also affected biochemical biomarkers of *G. pulex* in different levels.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

References

1. Lau ETC, Karraker NE, Leung KMY (2015) Temperature-dependent acute toxicity of methomyl pesticide on larvae of 3 Asian amphibian species. Environ Toxicol Chem 34(10):2322–2327
2. Mansour SA, Abdel-Tawab HM (2009) Tarek Effects of methomyl on lipidperoxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. Toxicol Ind Health 25(8):557–563
3. Ferreira D, da Motta AC, Kreutz LC, Toni C, Loro VL, Barcellos LJ (2010) Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. Chemosphere 79(9):914–921
4. Limón-Pacheco J, Gonsebatt ME (2009) The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res 674(12):137–147
5. Łaszczyca P, Augustyniak M, Babczyńska A, Bednarska K, Kafel A, Migula P, Wilczek G, Witas I (2004) Profiles of enzymatic activity in earthworms from zinc, lead and cadmium polluted areas near Olkusz (Poland). Environ Int 30:901–910
6. Schreck E, Geret F, Gontier L, Trilhou M (2008) Neurotoxic effectand metabolic responses induced by a mixture of six pesticideson the earthworm *Aporrectodea caliginosa nocturna*. Chemosphere 71:1832–1839
7. Vos RME, van Bladeren PJ (1990) Glutathione S-transferases in relation to their role in the biotransformation of xenobiotics. Chem Biol Interact 75:241–265
8. Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW (2004) Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics 14:1–18
9. Zama D, Meraihi Z, Tebibel S, Benayssa W, Benayache F, Benayache S, Vlitinck AJ (2007) Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats: the protective role of the butanolic extract of *Paronychie argentea* L. Indian. Pharmacogenetics 39:145–150
10. Adam O, Degiorgi F, Crini G, Badot PM (2010) High sensitivity of *Gammarus* sp. juveniles to deltamethrin: outcomes for risk assessment. Ecotoxicol Environ Saf 73:1402–1407
11. De Lange HJ, Noordoven W, Murk AJ, Lürling MFLLW, Peeters ETHM (2006) Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat Toxicol 78:209–216
12. Zelles L, Adrian P, Bai QY, Stepper K, Adrian MV, Fischer K, Maier A, Ziegler A (1991) Microbial activity measured in soils stored under different temperature and humidity conditions. Soil Biol Biochem 23:955–962
13. Apha AWWA, WPCF (2005) American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington
14. Arias-Estévez M, López-Periago JE, Martínez CE, Gandara JS, Mejuto JC, Garcia RL (2008) The mobility and degradation of

pesticides in soils and the pollution of groundwater resources. Agr Ecosyst Environ 123(4):247–260
15. Erguven GO, Yildirim N (2016) Efficiency of some soil bacteria for chemical oxygen demand reduction of synthetic chlorsulfuron solutions under agiated culture conditions. Cell Mol Biol 62(6):92–96
16. Erguven GO, Yildirim N (2019) The evaluation of imidacloprid remediation in soil media by two bacterial strains. Curr Microbiol 76(12):1461–1466
17. Lakshmi CV, Kumar M, Khanna S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeterior Biodegrad 62(2):204–209
18. Basak B, Bhunia B, Dey A (2014) Studies on the potential use of sugarcane bagasse as carrier matrix for immobilization of Candida tropicalis PHB5 for phenol biodegradation. Int Biodeterior Biodegrad 93:107–117
19. Lin J, Gan L, Chen Z, Naidu R (2015) Biodegradation of tetradecane using *Acinetobacter venetianus* immobilized on bagasse. Biochem Eng J 100:76–82
20. Jancova P, Anzenbacher P, Anzenbacherova E (2010) Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154(2):103–116
21. Bajda S, Dermauw W, Greenhalgh R, Nauen R, Tirry L, Clark RM, Leeuwen TV (2015) Transcriptome profiling of a spirodiclofen susceptible and resistant strain of the European red mite Panonychus ulmi using strand-specific RNA-seq. BMC Genomics 16:924
22. Andersson T, Pesonen M, Johansson C (1985) Differential induction of cytochrome P-450 dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP-glucuronosyltransferase activities in the liver of the rainbow trout by β-naphthoflavone or Clophen A50. Biochem Pharmacol 34:3309
23. Mansour SA, Mossa ATH, Heikal TK (2009) Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: In vitro studies. Toxicol Ind Health 25(8):557–563
24. Moreno DH, Resino IDLC, Flores JM, Gómez MJL, Neila CM, Soler F, López MP (2014) Different enzymatic activities in carp (*Cyprinus carpio* L.) as potential biomarkers of exposure to the pesticide methomyl. Arh Hig Rada Toksikol 65:311–318
25. Serdar O (2019) The effect of dimethoate pesticide on some biochemical biomarkers in Gammarus pulex. Environ Sci Pollut Res 26(21):21905–21914
26. El-Demerdash F, Azza AA, Reda H (2012) Elmazoudy biochemical and histopathological changes ınduced by different time intervals of methomyl treatment in mice liver. J Environ Sci Health A 47:1948–1954
27. Patil J, Patil AJ, Sontakke AV, Govindwar SP (2008) Effect of methomyl on hepatic mixed function oxidases in rats. Indian J Pharmacol 40(4):158–163
28. Delescluse C, Ledirac N, Li R, Piechocki MP, Hines RN, Gidrol X, Rahmani R (2001) Induction of cytochrome P450 1A1 gene expression, oxidative stress, and genotoxicity by carbaryl and thiabendazole in transfected human HepG2 and lymphoblastoid cells. Biochem Pharmacol. 61(4):399–407
29. Timbrell J (2000) Principles of biochemical toxicology, 3rd edn. Taylor and Francis, London
30. Heikal TM, Mossa A-T, Khalil WKB (2014) Protective effects of vitamin C against methomyl-induced injures on the testicular antioxidant status and apoptosis-related gene expression in rat. J Environ Anal Toxicol 5(2):1000255
31. Yildirim NC, Tanyol M, Yildirim N, Serdar O, Tatar S (2018) Biochemical responses of Gammarus pulex to malachite green solutions decolorized by Coriolus versicolor as a biosorbent under batch adsorption conditions optimized with response surface methodology. Ecotoxicol Environ Saf 156:41–47.