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ORIGINAL ARTICLE

Determination of selected pesticides in water samples adjacent to agricultural fields and removal of organophosphorus insecticide chlorpyrifos using soil bacterial isolates

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Abstract The use of pesticide for crops leads to serious environmental pollution, therefore, it is essential to monitor and develop approaches to remove pesticide from contaminated environment. In this study, water samples were collected to monitor pesticide residues, and degradation of chlorpyrifos was also performed using soil bacteria. Identification of pesticide residues and determination of their levels were performed by high-performance liquid chromatography with photodiode array detector. Among 12 samples, 10 samples were found contaminated with pesticides. Chlorpyrifos was detected in four tested samples and concentrations ranged from 3.27 to 9.31 µg/l whereas fenitrothion ranging from (Below Detection Limit, <0.1 µg/l) to 33.41 µg/l in the tested samples. Parathion was found in two tested samples at the concentration of 0.73 and 6.23 µg/l. None of the tested samples was found contaminated with Methoxychlor, DDT and Ethion. Three soil bacterial isolates, *Pseudomonas peli* BG1, *Burkholderia caryophylli* BG4 and *Brevundimonas diminuta* PD6 degraded chlorpyrifos completely in 8, 10 and 10 days, respectively, when 20 mg/l chlorpyrifos was supplied as sole source of carbon. Whereas, BG1, BG4 and PD6 took 14, 16 and 16 days, respectively, for complete removal of 50 mg/l chlorpyrifos. Chlorpyrifos degradation rates were found maximum by all three isolates at 2nd day of incubation for both tested concentrations. The results of the present study suggest the need for regular monitoring of pesticide residues in water, to protect the aquatic environment. Chlorpyrifos degrading bacterial isolates can be used to clean up environmental samples contaminated with the organophosphate pesticides.

Keywords Pesticides · Agricultural water · HPLC · Organophosphorus insecticide · Chlorpyrifos · Biodegradation · Bangladesh

Introduction

Agriculture is the main economic backbone of Bangladesh, which contributes about one-third to the country's gross domestic product (GDP) and 80 % of the people depend on agriculture for their livelihood (Chowdhury et al. 2011; Bhattacharjee et al. 2012). However, agricultural production has increased in recent years with the introduction of high-yielding cultivars of rice. But these high-yielding cultivars of rice are highly vulnerable to pests and diseases and dramatically reduce agricultural output. Therefore, like many other developing countries, pesticides are used extensively in Bangladesh to increase the crop yield (Shahjahan 1993; Dasgupta et al. 2007; Chowdhury et al. 2012b). Due to the widespread use of pesticides, their residues are detected in various environmental matrices, like soil, water and air. Pesticide contaminations of surface waters have been well documented worldwide and cause a major issue that gives rise to concerns at local, regional, national and global scales due to the adverse effects of pesticide on the environment (Planas

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et al. 1997; USGS 1999; Huber et al. 2000; Cerejeira et al. 2003). Pesticide residues reach the water body through direct runoff, leaching, careless disposal of empty containers, equipment washing etc. The amount of pesticides lost from agricultural fields and transported to surface waters depends on several factors, including soil characteristics, topography, weather, agricultural practices, and chemical and environmental properties of individual pesticides (Wagenet 1987; Leonard 1990). Chemical reactions and physical displacements influence the persistence of the chemicals in the soil and water, but with different environmental implications. Pesticides, which are sufficiently resistant to degradation and are adequately soluble to be transported in water, may reach the water bodies in significant amounts (Wagenet 1987).

For the treatment of water polluted with pesticides, many technologies have been evaluated including physicochemical and biological treatments. Biological treatment like biodegradation of pesticide using microorganisms has received increasing attention as an efficient, inexpensive and environmentally friendly approach to clean up pesticide-polluted environments (Xu et al. 2008). Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is used worldwide as an agricultural insecticide (Cho et al. 2002). There are numerous studies of chlorpyrifos insecticide degradation by microorganisms (Singh et al. 2004; Lakshmi et al. 2008; Anwar et al. 2009; Briceño et al. 2012). The aim of this paper is to determine the concentration of the selected pesticide residues in agricultural water samples of Savar Upazila and to degrade chlorpyrifos using soil bacteria.

Materials and methods

Standard pesticides and chemicals

Reference grade standards for methoxychlor (99.5 %), DDT (99.0 %), chlorpyrifos (99.5 %), diazinon (99.5 %), ethion (99.0 %), fenthion (98.5 %), fenitrothion (99.0 %), malathion (99.5 %), parathion (99.0 %), carbaryl (99.0 %), carbofuran (99.5 %) and cypermethrin (99.0 %) were purchased from GmbH (D-86199 Augsburg, Germany). The organic solvents including n-hexane (Merck, Germany), diethylether (BDH, England) and dichloromethane (Merck, Germany) were of analytical grade while acetonitrile (Scharlau, EU) was of HPLC grade. Anhydrous Sodium Sulfate (Merck, Germany), Florisil (Sigma, USA) and formulated chlorpyrifos (Master Plus) were also used.

Collection and preservation of water samples

Water samples from lakes adjacent to agricultural fields of Savar, Bangladesh were collected from February to March, 2012. The samples were kept in clean amber glass bottles,

put into ice boxes and immediately transferred to the laboratory at the Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka. Sample collection was performed according to the recommendations by Hunt and Wilson (1986) and APHA (1995). The samples were properly labeled and kept at −20 °C before analysis according to Uddin et al. (2007).

Water sample processing

Five hundred milliliter of water sample and 100 ml solvent (2 % diethyl ether in double-distilled n-hexane) were taken into a 1,000-ml capacity separating funnel (DURAN, Germany) and was shaken by mixing well for about 10 min and then kept standing for 10–15 min for settling down. The organic solvent was collected in a conical flask. The aqueous layer was re-extracted by adding 50 ml of solvent mixture using a similar procedure. The organic solvent layers were aspirated and combined before the addition of 20 g of anhydrous sodium sulfate (Merck, Germany) to remove the residual water. The collected extract was then concentrated to a smaller volume of 5 ml under reduced pressure using a rotary vacuum evaporator (Rotavapor-R 215, Buchi, Switzerland) based on the method described in the Deutsche Forschungsgemeinschaft (Thier and Zeumer 1987). The samples were cleaned up by following the method described by (Bhattacharjee et al. 2012). In brief, the concentrated samples were passed through a column (10 mm ID) packed with 10 g of deactivated florisil (Sigma, USA) and synthetic magnesium silicate (60–100 mesh). The florisil was activated at 200 °C for 6 h and was subsequently deactivated with 2 % distilled water. The top 1.5 cm of the florisil column was packed with anhydrous sodium sulfate. Elution was performed with a solvent mixture of double-distilled hexane (90 %) and dichloromethane (10 %) at 5 ml/min. The eluent was further concentrated in a rotary vacuum evaporator (Buchi, Switzerland) before being transferred to a glass vial. Solvents were completely removed under a gentle flow of fresh nitrogen from a Nitrogen blower (PU 90003, ALFA industry, England). The evaporated sample was dissolved in acetonitrile, and a 1-ml volume was used for the HPLC analysis.

Pesticide analysis by HPLC

After the sample cleanup, aliquots of the final volume were quantified with a HPLC system (SHIMADZU LC-10 Avp-Series Automated with LC Solution Software LabSolutions (LC solution Release 1.11SP1) that was equipped with a SPD-M 10 Avp outfitted with a photodiode array (PDA) detector. A C_{18} Reverse Phase Alltech analytical column (5 μm, 250 9 4.6 mm) was used and

maintained at 30 °C in a column oven. The mobile phase, which was a combination of 70 % acetonitrile and 30 % water, was filtered using a cellulose filter (0.45 μm) prior to use and was allowed to run at 1.2 ml/min. Prior to the HPLC analysis, the samples were passed through 0.45 μm nylon (Alltech Assoc) syringe filters and were manually injected (20 μl) into the HPLC system each time. The identification of the suspected pesticide was performed by comparing peak retention times in samples to those of peaks in the pure analytical standard. Quantification was performed using the method described by (Chowdhury et al. 2012a).

The percentage recoveries were calculated using the following equation

Percentage of recovery $= [C_E/C_M \times 100]$, where C_E is the experimental concentration determined from the calibration curve and C_M is the spiked concentration.

Table 1 Percentage recoveries of tested pesticides for water samples

Compound	Cleanup system	Concentration (µg/l)[a]		Recovery %
		Spiked	Measured	
Methoxychlor	Control[b]	0.00	0.00	0.00
	Florisil cleanup	400.00	357.20	89.30
DDT	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	365.80	91.45
Chlorpyrifos	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	378.16	94.54
Diazinon	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	353.28	88.32
Ethion	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	372.48	93.12
Fenthion	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	343.92	85.98
Fenitrothion	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	357.00	89.25
Malathion	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	366.48	91.62
Parathion	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	361.64	90.41
Carbaryl	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	370.04	92.51
Carbofuran	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	373.64	93.41
Cypermethrin	Control	0.00	0.00	0.00
	Florisil cleanup	400.00	349.28	87.32

[a] Mean value of triplicates

[b] Control is blank sample without spiking of any concentration of pesticides

The mean percentage recoveries of all tested pesticides were more than 85 % which was satisfactory. These values were quite satisfactory and meet the requirements of the (European Commission 2000) (Tables 1), indicating that a method can be considered accurate and precise when the accuracy of data is between 70 and 110 %. The Limit of detection (LOD) for the pesticides was 0.1 µg/l.

Bacteria used in this study

Three bacterial isolates viz., *Pseudomonas peli* BG1, *Burkholderia caryophylli* BG4 and *Brevundimonas diminuta* PD6 were collected from Microbiology and Industrial Irradiation Division, Institute of Food and Radiation Biology (IFRB), Atomic energy Research Establishment, Savar, Dhaka. The organisms were isolated from botanical garden of Jahangirnagar University campus in October 2011 and identified by Mahiuddin (2012).

Growth medium and biodegradation studies

The mineral salt medium used in biodegradation studies, adapted from Cycon et al. (2009), contained (g/l) $(NH_4)_2SO_4$, 2.0; KH_2PO_4, 1.5; Na_2HPO_4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.001. The pH of the medium was adjusted to 7.0 ± 0.1 with 2 M NaOH. Growth experiments with chlorpyrifos as a sole carbon source were performed in 250-ml conical flasks containing 95 ml of sterile mineral salt medium (MSM). Chlorpyrifos was introduced into MSM prior to inoculation in a form of acetone solution to give the final concentration of 20 and 50 mg/l. After a day of shaking to evaporate the solvent, 5 ml of bacterial suspension ($\approx 10^7$) was used to inoculate in 95 ml sterile mineral salt medium. Mineral salt medium was sterilized by autoclaving prior to the addition of chlorpyrifos. Triplicate samples for MSM + chlorpyrifos, non-inoculated with the bacterial suspension were kept as controls. After inoculation, flasks were incubated at 30 °C on a rotary shaker (120 rpm) and kept from the light to avoid photodegradation of chlorpyrifos.

Samples of liquid medium were periodically removed aseptically for measuring of bacterial growth ($OD_{600 nm}$) and pH using UV–visible spectrophotometer (UV-1601, SHIMADZU) and pH meter (3510 pH meter, JENWAY, Bibby Scientific Ltd., UK), respectively. A portion (~ 5 ml) of liquid medium from conical flasks was taken aseptically in centrifuge tube and cells were removed by centrifugation at 8,000 rpm for 10 min. After that the supernatant was taken and allowed to centrifuge again at the same condition. The final supernatant (~ 1 ml) was taken into eppendorf and kept in refrigerator prior to their analysis using HPLC. The study period for the biodegradation of chlorpyrifos was up to 12 days when 20 mg/l

chlorpyrifos was supplied as a sole source of carbon and up to 16 days when 50 mg/l chlorpyrifos was supplied as a sole source of carbon.

Determination of degradation rate

The degradation rate by the bacterial isolate under specific conditions was calculated as the following equation: Degradation rate $= \Delta$ [Chlorpyrifos]$/\Delta$ [t],

where, Δ [Chlorpyrifos] was the difference of chlorpyrifos concentrations between two sampling times (mg/l) and Δ [t] was time difference in days.

Results and discussion

Level of organochlorines, organophosphorus, carbamates and pyrithrium pesticide residues in agricultural water samples

Twelve water samples from Savar Upazila of Dhaka, Bangladesh were analyzed for some selected organochlorines (OCs), organophosphorus (OPs), carbamates and pyrithrium pesticides using high-performance liquid chromatography (HPLC) with PDA detector. Table 2 presents the results of pesticide residues (µg/l) in water samples. Among organophosphorus pesticides, chlorpyrifos was detected in four samples and concentrations ranged from 3.27 to 9.31 µg/l, which was lower than the standard value of Japan (MPHW 1997) whereas diazinon was detected in only one sample at the concentration of 7.86 µg/l, which was much higher than the Australian Health Value of 3 µg/ l for diazinon (NHMRC 1996). On the other hand, fenitrothion was detected and concentrations ranged from 5.83 to 33.41 µg/l, which was below than the Australian Health Value of 10 µg/l (NHMRC 1996). Parathion was found and concentration ranged from 0.73 to 6.23 µg/l, below the Australian Health Value of 10 µg/l (NHMRC 1996) whereas malathion was detected and concentrations ranged from 23.1 to 59.9 µg/l, within the Australian Health Value (NHMRC 1996). In this study, ethion was not detected in any of the tested samples whereas fenthion was detected in two samples at the concentration of 25.11 and 56.3 µg/l, respectively. Previously investigated diazinon concentrations ranged from no detected to 0.102 µg/l in Axios Greek River (Papadopoulou-Mourkidou 2002), fenitrothion <1 µg/l (Sudo et al. 2002), parathion methyl from no detected to 271 ng/l and parathion ethyl from no detected to 40 ng/l in Kalamas Greek River (Albanis et al. 2004), fenthion residues ranged from 2.2 to 15 µg/l in Kenya (Keith et al. 1994) and 10–30 ng/l in Kalamas River (Lambropoulou et al. 2002). The persistent nature of organophosphorus pesticides and their translocation from agricultural land as runoff or as irrigation return flow can cause residues in water sources.

Among carbamate pesticides, carbaryl was determined and concentrations ranged from 4.6 to 6.3 µg/l, whereas carbofuran was detected at a concentration of 43.2 µg/l. The values of carbaryl were almost similar to the Australian Guideline Value of 5 µg/l whereas carbofuran was well above the Australian Guideline Value of 5 µg/l (NHMRC (National Health and Medical Research Council) 1996). Carbaryl was detected in Evros basin from 0.046 to 0.077 µg/l (Vryzas et al. 2009). Previously recorded carbofuran ranged from no detected to 160 ng/l in Kalamas River and no detected to 553 ng/l in Arachthos River in Greece (Albanis et al. 2004), and 30–150 ng/l from Kalamas River (Lambropoulou et al. 2002).

In this study, cypermethrin was detected in three of the tested samples and concentrations ranged from 54.36 to 80.5 µg/l, which are very higher than the Swedish guideline value of 0.0002 µg/l for cypermethrin and EU regulatory value of 0.0068 µg/l for cypermethrin in stagnant water systems (Wijngaarden et al. 2005). Cypermethrin as a hydrophobic organic compound which adsorbs to sediments and according to the literature it is toxic to aquatic organisms (Tomlin 2010). On the other hand, once they have been adsorbed to sediment, they can leach and cause long-term contamination of watercourses (Turgut et al. 2010). Major reported sources of cypermethrin in surface water include agricultural and urban runoff from rainstorms, spray drift, urban landscape irrigation and release of agricultural tailwaters (Wang et al. 2009).

The results of the present study showed that none of the water samples was contaminated with organochlorine (methoxychlor and DDT) pesticides. These may be the ban of organochlorines from the pesticide markets from 1993 according to the Bangladesh Environment Conservation Act 1995 due to their high toxicity (Matin et al. 1998). Another study conducted on the presence of organochlorine residues in water samples from irrigation canals in the Meghna-Dhanogoda irrigation project, Bangladesh, reported that the maximum amount of DDT was 5.7 µg/l (Alam et al. 1999), but in the present study, no DDT was detected in water. Matin et al. (1998) also reported that DDT was present in water from irrigated crop field 1.5 µg/l in Gaibanda and 19.6 µg/l in Begumganj. Golfinopoulos et al. (2003) reported that methoxychlor remains below detection limit in water samples from various lakes at different sampling periods.

The frequent high concentrations (over guideline values) of pesticides detected in the current study are probably due to the extensive utilization of pesticides in the agricultural fields. There are three major sources for surface water contamination by pesticides: inappropriate handling of pesticides (e.g., washing equipment in the river),

Table 2 Pesticide residues (µg/l) in water samples collected from near of the vegetable fields of Savar Upazila

Sample ID	Concentration (µg/l)[a]											
	Methoxychlor	DDT	Chlorpyrifos	Diazinon	Ethion	Fenthion	Fenitrothion	Malathion	Parathion	Carbaryl	Carbofuran	Cypermethrin
WS-1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	23.1	BDL	BDL	BDL	BDL
WS-2	BDL	BDL	4.13	BDL	BDL	BDL	BDL	BDL	BDL	BDL	43.2	BDL
WS-3	BDL	BDL	BDL	7.86	BDL	BDL	19.4	BDL	BDL	4.6	BDL	80.5
WS-4	BDL	BDL	9.31	BDL	BDL	25.11	BDL	BDL	BDL	BDL	BDL	BDL
WS-5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	59.9	0.73	BDL	BDL	BDL
WS-6	BDL	BDL	5.3	BDL	BDL	BDL	5.83	BDL	6.23	6.3	BDL	BDL
WS-7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
WS-8	BDL	BDL	BDL	BDL	BDL	BDL	33.41	BDL	BDL	BDL	BDL	74.62
WS-9	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
WS-10	BDL	BDL	3.27	BDL	BDL	BDL	14.2	BDL	BDL	BDL	BDL	BDL
WS-11	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	54.36
WS-12	BDL	BDL	BDL	BDL	BDL	56.3	BDL	BDL	BDL	5.27	BDL	BDL

Detection limit = 0.1 µg/l

WS water sample, *BDL* below detection limit

[a] Mean value of triplicates

volatilization to air, and residuals from soil and water. In this perspective, the drainage of water from agricultural fields into irrigation channels poses a critical source of risk for contamination by pesticides in the broader water environment (Phong et al. 2010). Rain runoff from agricultural fields that occurred immediately after the application period of pesticides has been pointed to be the major source of pesticides in water bodies (Kreuger 1998). Rainfall, and hence the associated increased runoff, would be expected to result in increased movement of pesticides into nearby water bodies. However, the persistent nature of the pesticides is of great concern due to their bioaccumulative nature and toxic biological effects on wildlife and humans (Tanabe et al. 2000).

Biodegradation of chlorpyrifos by three bacterial strains

Earlier attempts to degrade chlorpyrifos by soil bacterial isolates have not been successful before 2000 (Racke et al. 1994; Mallick et al. 1999). In the present study, biodegradation of chlorpyrifos by three soil bacterial isolates was performed in comparison with bacterial cell growth in the MSM. Growth of bacterial isolates, chlorpyrifos degradation and change in pH by *P. peli* BG1 when 20 mg/l chlorpyrifos and 50 mg/l chlorpyrifos were supplied as sole source carbon, are presented in Fig. 1. The growth of *P. peli* BG1 reached a maximum OD_{600} at 6 and 8 days of incubation in 20 mg/l chlorpyrifos and 50 mg/l chlorpyrifos, respectively. The results showed that *P. peli* BG1 degrades chlorpyrifos completely within 8 and 14 days of incubation when 20 and 50 mg/l chlorpyrifos were supplied as sole source of carbon, respectively. But the bacterial growth and chlorpyrifos degradation were both increased rapidly during 2 days of incubation in chlorpyrifos-containing medium. Result also represented that, when growth of the bacterial cell increased, chlorpyrifos degradation also relatively increased, similar results also found in other studies (Xu et al. 2007, 2008). Xu et al. (2007) observed the complete disappearance of 50 mg/l chlorpyrifos due to *Serratia* sp. TCR cultivated in mineral salt medium within 4 days. Similar results were recorded by Zhu et al. (2010) and 100 mg/l of chlorpyrifos was completely degraded by *B. licheniformis* ZHU-1 within 14 days. At 25 and 50 mg/l chlorpyrifos, maximum microbial growth was attained on the 5th day and thereafter growth declined during degradation of chlorpyrifos by *Pseudomonas aeruginosa* (Huang et al. 2000). Li et al. (2008) reported that approximately 98 % of chlorpyrifos was degraded within 10 days by seven bacterial isolates belonging to *Pseudomonas* genus collected from soil.

Figure 2 presents the both bacterial growth and degradation of chlorpyrifos for the isolate *B. caryophylli* BG4 when 20 and 50 mg/l chlorpyrifos were used, respectively,
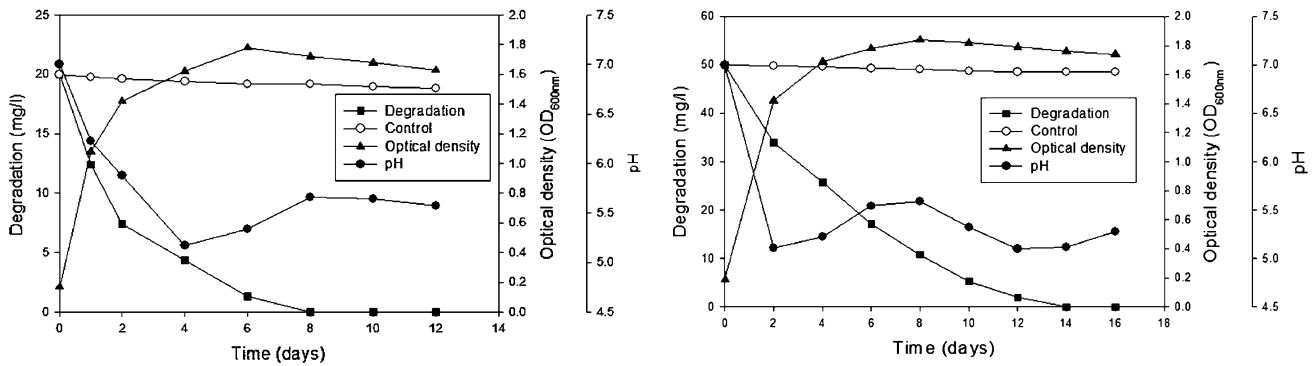
Fig. 1 Chlorpyrifos degradation, growth and change in pH by *Pseudomonas peli* BG1 when 20 and 50 mg/l chlorpyrifos were supplied as sole source of carbon
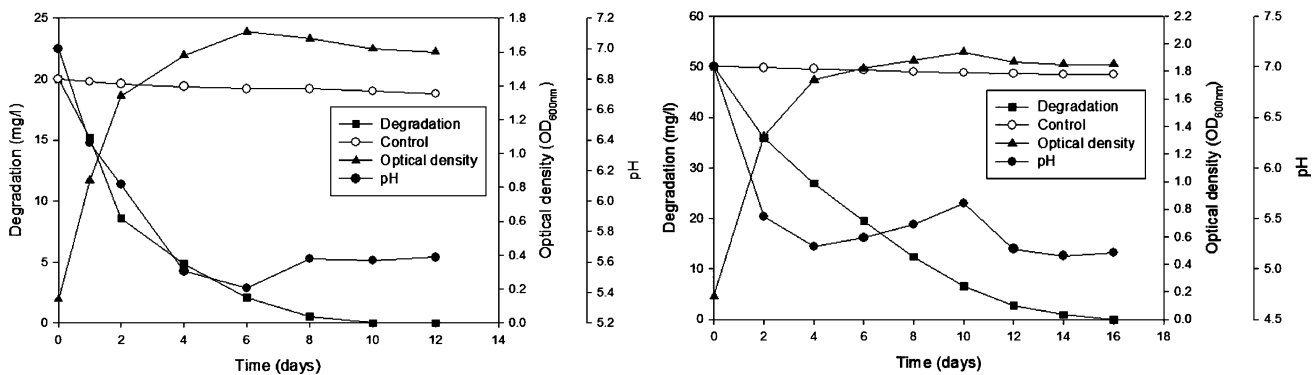
Fig. 2 Chlorpyrifos degradation, growth and change in pH by *Burkholderia caryophylli* BG4 when 20 and 50 mg/l chlorpyrifos were supplied as sole source of carbon
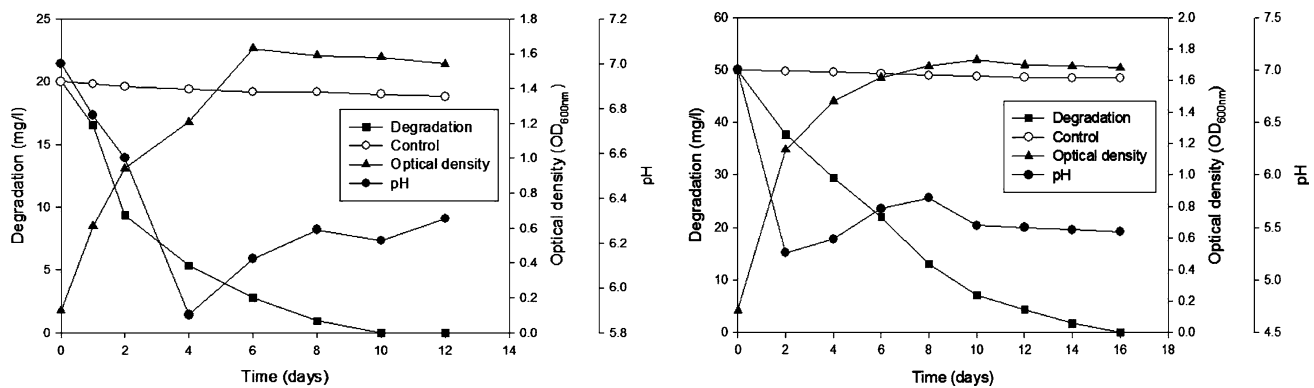
Fig. 3 Chlorpyrifos degradation, growth and change in pH by *Brevundimonas diminuta* PD6 when 20 and 50 mg/l chlorpyrifos were supplied as sole source of carbon

as sole carbon source, respectively. The cell growth reached a maximum after 6 (20 mg/l) days and 10 (50 mg/l) days of incubation and chlorpyrifos was completely degraded within 10 and 16 days of incubation in 20 and 50 mg/l chlorpyrifos-containing medium, respectively. The pH range was 7–5.43 during degradation of chlorpyrifos at the concentration of 20 mg/l and 7–5.13 when concentration was 50 mg/l. The results revealed that pH drastically decline in 2 days of incubation and maximum degradation

was found in 50 mg/l of chlorpyrifos. The isolated *Enterobacter* species were capable of degrading chlorpyrifos in the pH range from 5.5 to 7.6 (Singh et al. 2004). Kim and Ahn (2009) were examined the degradation of different insecticides in MSM cultures using *Burkholderia* sp. strain KR100 cell extract.

For the isolate *B. diminuta* PD6, the results of bacterial growth and degradation of chlorpyrifos are presented in Fig. 3 when 20 and 50 mg/l chlorpyrifos were supplied as

sole source of carbon, respectively. The results showed that growth of bacterial cell was reached a maximum after 6 (20 mg/l) days and 10 (50 mg/l) days of incubation and chlorpyrifos was completely degraded within 10 and 16 days of incubation in 20 mg/l chlorpyrifos and 50 mg/l chlorpyrifos-containing medium, respectively. The growth of the isolate *B. diminuta* PD6 was not increased after complete degradation of chlorpyrifos. The pH range was 7–5.88 during degradation of chlorpyrifos by *B. diminuta* at the concentration of 20 mg/l and 7–5.26 when concentration was 50 mg/l. The results also revealed that pH rapidly decline in 2 days of incubation and maximum degradation was found in 50 mg/l of chlorpyrifos. Maximum chlorpyrifos degradation rate by *B. diminuta* PD6 was found during 2 days of incubation in both 20 mg/l chlorpyrifos and 50 mg/l chlorpyrifos-containing medium. Previously Desdpande (2002) isolated two strains belonged to the genus *Brevundimonas* and identified as *Brevundimonas* sp. to study the effect of various environmental factors on growth and dimethoate (an organophosphorus insecticide) degradation; the isolate *Brevundimonas* sp. was grown in Davis Mingiolo's synthetic medium containing 500 mg/l of dimethoate as sole source of carbon and energy.

Though a little information exists regarding the ability to degrade chlorpyrifos by bacteria belonging to *Burkholderia* and *Brevundimonas* species, some studies showed a lot of information about the efficient degradation of chlorpyrifos performed by the different species of *Pseudomonas* (Digrak et al. 1995; Huang et al. 2000; Li et al. 2008; Rani et al. 2008; Xu et al. 2008; Liu et al. 2012). Chlorpyrifos has also been reported to be degraded co-metabolically by bacteria, which needs extra carbon sources (Richins et al. 1997; Mallick et al. 1999). Singh et al. (2004) reported isolation of *Enterobacter* sp. B14, which can use chlorpyrifos for the supply of carbon and

phosphorous sources, which it stopped degrading chlorpyrifos in the presence of other carbon sources. Recently, *Enterobacter* strain B-14 (Singh et al. 2003), *Alcaligenes faecalis* DSP3 (Yang et al. 2005) and *Stenotrophomonas* sp. (Yang et al. 2006; Lakshmi et al. 2008) were isolated and showed an enhanced degradation of chlorpyrifos.

Chlorpyrifos biodegradation rate

The degradation rate patterns of chlorpyrifos in MSM supplemented with 20 and 50 mg/l chlorpyrifos are presented in Fig. 4. It was observed that the chlorpyrifos degradation rate for these three isolates was different at the same incubation period when grown in MSM supplemented with 20 and 50 mg/l chlorpyrifos. Maximum chlorpyrifos degradation rates by all three isolates were found in 2nd day of incubation for both 20 and 50 mg/l chlorpyrifos. From the results it was also found that the degradation rate was relatively higher for all isolates during first 6 days of incubation but then it decreased sharply. During the first 2 days of incubation period, chlorpyrifos was degraded at a maximum rate of 6.3, 5.7 and 5.3 mg/l/ day for isolates BG1, BG4 and PD6, respectively, when MSM supplemented with 20 mg/l chlorpyrifos. On the other hand, the maximum degradation rates were obtained by the isolates BG1, BG4 and PD6 with 8.04, 7.04 and 6.14 mg/l/d, respectively, after 2 days of incubation when MSM supplemented with 50 mg/l chlorpyrifos.

In this study, it has been found that the chlorpyrifos degradation rates by the isolates *P. peli* BG1, *B. caryophylli* BG4 and *B. diminuta* PD6 were greatly increased due to increase in the concentration of chlorpyrifos; similar results were found in other studies (Li et al. 2008; Xu et al. 2008). Li et al. (2008) also showed that 100 mg/l chlorpyrifos was degraded to an undetectable level in 24 h by
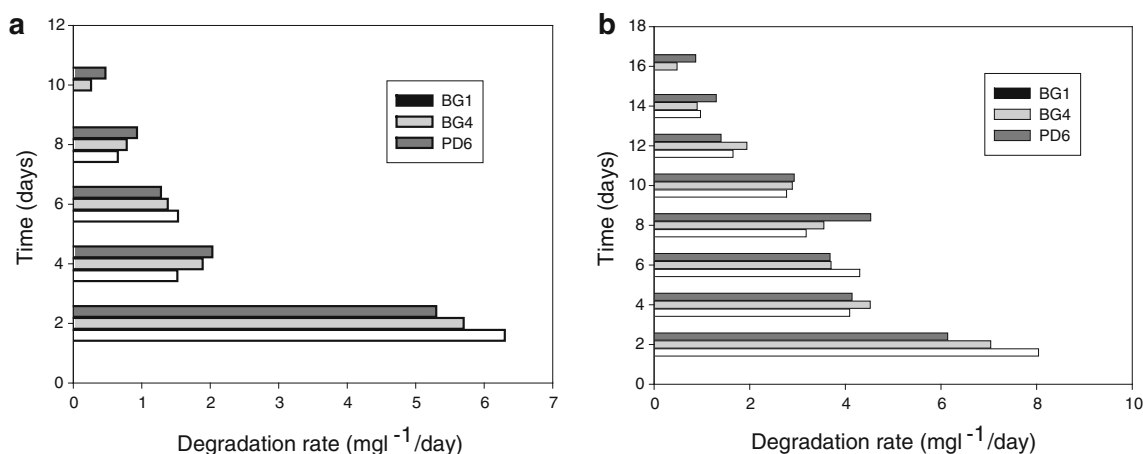
Fig. 4 Degradation rate of chlorpyrifos by the three isolates *Pseudomonas peli* BG1, *Burkholderia caryophylli* BG4 and *Brevundimonas diminuta* PD6 when grown in MSM containing **a** 20 mg/l and **b** 50 mg/l concentration of chlorpyrifos

strain Dsp-2 belonging *Pseudomonas* genus. Contrast of the results also demonstrated by Liu et al. (2012) that the degradation rate was decreased slowly with the concentration of chlorpyrifos being increased from 100 to 150 mg/l. The degradation of chlorpyrifos by *P. fluorescence*, *B. melitensis* and *B. subtilis* was 43, 45 and 56 %, respectively, after 10 days of incubation and 89, 87 and 85 %, respectively, after 30 days of incubation, when 50 mg/l chlorpyrifos was supplied as sole source of carbon (Lakshmi et al. 2008).

Conclusions

Surface waters adjacent to agricultural area of Savar Upazila were contaminated with pesticide residues, particularly chlorpyrifos, fenitrothion, fenthion, malathion, carbaryl and cypermethrin. Other pesticides detected were parathion, diazinon and carbofuran. In the tested samples, chlorpyrifos, fenitrothion and parathion were found ranged from 3.27 to 9.31 µg/l, 5.83 to 33.41 µg/l, and 0.73 to 6.23 µg/l, respectively. Carbaryl, cypermethrin and malathion were recorded ranged from 4.6 to 6.3 µg/l, 54.36 to 80.5 µg/l and 23.1 to 59.9 µg/l, respectively. None of the water sample was found to be contaminated with Methoxychlor, DDT and Ethion. Proper utilization and routine monitoring programs for these pesticide residues in agro-environment are needed to prevent, control and reduce the pollution.

Pseudomonas peli BG1, *B. caryophylli* BG4 and *B. diminuta* PD6 degraded chlorpyrifos completely in 8, 10 and 10 days of incubation, respectively, when 20 mg/l chlorpyrifos was supplied as sole source of carbon. On the other hand, BG1, BG4 and PD6 took 14, 16 and 16 days, respectively, when the concentration of chlorpyrifos was 50 mg/l. Maximum chlorpyrifos degradation rates were recorded by all three bacterial isolates at 2nd day of incubation for both tested concentrations of 20 and 50 mg/l. Isolates BG1, BG4 and PD6 can be used successfully for the bioremediation of chlorpyrifos-contaminated sites.

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