Impacts of Chlorpyrifos and Deltamethrin on Soil Bacterial Community Composition in Different Salinity Soils: Natural Attenuation Microcosm Studies

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Research Article

Keywords: Soil microbial diversity, Soil contamination, deltamethrin, chlorpyrifos, next generation sequencing, natural attenuation

Posted Date: June 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-175997/v1

Abstract

Pesticides and insecticides are the chemicals widely used in the agricultural industry, but their ecotoxicological effects on the environment are not still well understood. In this study, the remediation of chlorpyrifos (CP) and deltamethrin (DM) and their impacts on soil microbial diversity was investigated. Four different soils with various salinity (0%, 1%, 2% and 4%) were artificially contaminated by CP and DM. Then, natural attenuation of the pesticides in soil microcosms and their effects on soil microbial composition were studied by metagenomics. The pesticide natural attenuation analysis showed higher CP remediation in slightly saline soils with 1% and 2% salinity and faster removal of DM in 1% saline soil in comparison to non-saline control microcosm. The complete natural attenuation of the contaminants took around 60 days. The metagenomics analysis indicated that pesticide contamination had significant impacts on the soil flora and some dominant species in the control microcosm were completely eliminated by CP and DM. In addition, *Paenibacillus* (2% salinity and DM), *Bacillus* (4% salinity and CP), *Paeniclostridium* (1% salinity and DM) and *Lachnospiraceae* (1% salinity and CP) were the dominant genus by 77%, 50%, 41% and 39% relative abundances, respectively. At phylum level, the sequences belonged to *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were considerably enriched during natural attenuation of both DM and CP pesticides. Furthermore, Shannon and Simpson Indexes were identified more sensitive to the microbial community evenness, while, Chao1 and ACE indexes were changed by the community abundance. It was revealed that the highest negative impacts of deltamethrin and chlorpyrifos on the culturable and unculturable communities were related to the non-saline soil.

Highlights

- The impacts of the chlorpyrifos and deltamethrin on bacterial composition of four soils with different salinities was studied
- The natural attenuation of the pesticides was monitored until complete removal (60 days) and bacterial count was followed for 250 days
- The effects of soil salinity on chlorpyrifos and deltamethrin degradation was investigated and proved that slight salinity improves the remediation
- Metagenomic analysis showed that each soil sample develops its own specific bacterial flora according to the soil physic-chemical properties

1. Introduction

Organophosphorus and pyrethroid compounds, especially chlorpyrifos (CP) and deltamethrin (DM) are still used in the worldwide as common pesticides, respectively (Hinojosa et al. 2020; Li et al. 2008; Zhang et al. 2016; Shrivastava et al. 2011). Numerous studies have been conducted on the negative impacts of these toxic compounds on the human health (Carr et al. 2020; Petrovici et al. 2020; Hamada et al. 2020). In this context, it was stated that the genes expression of vascular endothelial growth factor receptor 1,

peroxisome proliferator activated receptor gamma, hypoxia-inducible factor 1-alpha and the *β*-subunit of human chorionic gonadotropin is reduced by CP exposure (Ridano et al. 2019).

Although extensive researches have been rendered on the harm of widespread organophosphorus and pyrethroid compounds on the human and animal health and also their remediation from soil or water, but only a few of these investigations have focused on their impacts on the soil microbial diversity (Wu et al. 2020; ul Haq et al. 2020; Pailan et al. 2015; Sirotkina, Lyagin, and Efremenko 2012; Hossain et al. 2013). Chlorpyrifos inhibits the soil microbial nitrification process which results in less movement and transport of mineral nitrogen because of higher NH_4/NO_3 ratio in soils caused by retardation of nitrification (Kollah et al. 2019). It was found that the physiochemical properties of soil changed the toxicity of the CP and DM for the soil fauna (Jaabiri Kamoun et al. 2018). In addition, some studies investigated the removal of organophosphorus compounds from different media by novel remediation approaches; including soil biotreatment by native bacteria, physical adsorption using metal organic frameworks (MOFs) and phytoremediation process (Jacob et al. 2020; Rosbero and Camacho 2017; Hassan et al. 2020; Yang et al. 2017; Lin and Chuanling 2008).

The effect of soil chlorpyrifos-contamination on the microbial diversity was investigated and revealed that a *Streptomyces* strain was able to biotransform CP into lower dangerous compounds of 3, 5, 6-trichloro-2-pyridinol and diethyl phosphorothioate (Supreeth et al. 2016). In addition, the considerable role of CP-degrading bacteria on plant growth in a contaminated fields was studied (Akbar and Sultan 2016). It was indicated that soil microbial functional diversity is affected by chlorpyrifos residues in soil (Hua et al. 2009). Meanwhile, it was found that repeated application of chlorpyrifos increases its degradation rate by adaptation of soil microbial communities to this contaminant. Therefore, the inhibitory impact of chlorpyrifos on the microorganisms was significantly decreased (Fang et al. 2008).

Deltamethrin has inhibitory effect on the soil respiration under anaerobic condition, while, in the presence of nitrate this effect was greatly controlled (Munoz-Leoz et al. 2009). In contrast to these results, it was showed that DM has no considerable impact on the sediments community respiration (Widenfalk, Svensson, and Goedkoop 2004). Additionally, contamination of soil by deltamethrin results in the increased diversity and abundance of DM-degrading microorganisms (Dou et al. 2020). The isolated pyrethroid-degrading strains and their degradation pathways was recently reviewed, but, it was confessed that reaching an efficient bioremediation strategy is hardly rely on performing a comprehensive investigation on soil pyrethroid-degrading microorganisms (Zhan et al. 2020).

Several attempts have been made to remediate these contaminants from soil, and water via physical, chemical and biological approaches (Kapoor and Rajagopal 2011). The biological methods are proved to be the most efficient and economic candidates for removing or degrading organophosphorus and pyrethroid compounds from different media (Wang et al. 2020; Graça et al. 2020; Abdi et al. 2020; Bhat et al. 2020; Zhan et al. 2020; Wang et al. 2017; Xu et al. 2008). This process is performed through bioaugmentation (supplementing pollutant degrading microorganisms to soil) or biostimulation (addition of nutrients to stimulate soil natural flora) (Korade and Fulekar 2009; Tortella et al. 2010).

Within the diverse investigations which identified the factors pH (Yang et al. 2006), temperature (Das and Adhya 2015), contaminant concentration (Singh et al. 2003) and nutrients (Yadav et al. 2016) which affect the pesticides natural attenuation, none have addressed the soil salinity as an effective factor (Phogat et al. 2020; Tomaz et al. 2020). Collectively, no enough comprehensive researches have been found that surveyed the soil biodiversity after contamination by organophosphorus and pyrethroid compounds. The main purpose of this study was to investigate the effect of pesticide contamination on microbial diversity of saline soils under different salinity using next generation sequencing (NGS) in order to identify the most important pesticide-degrading bacteria in different soil samples.

2. Materials And Methods

2.1. Sampling and chemical analysis

Soil samples were collected from different areas of agricultural fields in Eshtehard, Alborz Province, Iran (Table S1, Supplementary Materials). Four samples including: sample 1 (35.743453, 50. 616550), sample 2 (35.743931, 50. 615236), sample 3 (35.744622, 50. 613446) and sample 4 (35.731582, 50. 614329) with 1%, 2%, 4% and 0% salinity, respectively were prepared. To get a representative samples, composite sampling method was used. Ten sub-samples were collected from a radius of one meter at each sampling point and mixed.

The physiochemical characteristics of the samples were analyzed by standard soil analysis procedures (Emadi et al. 2008; Gokalp et al. 2010). The salinity percentage of each soil sample was calculated and experimentally measured by a handheld refractometer (Mettler Toledo, China) (Corwin and Yemoto 2017). The results are presented in Table S2 (Supplementary Materials).

Table 1 indicates the physical and chemical properties of the soil samples. All the soil samples were slightly alkaline in nature with an average pH of 8. The texture of all soil samples were loamy sand having about 30% sand and 40–50% silt. As previously mentioned, the sample 3 showed higher salinity which was proved by higher electrical conductivity (EC).

2.2. Experimental microcosms

To prepare the soil remediation microcosms, 1 kg of sieved (2 mm) and air dried soil sample was spiked with acetone dissolved deltamethrin or chlorpyrifos (200 mg/kg soil) and mixed thoroughly. In order to evaporate the solvent, spiked soils were stored in the fume hood for 48 hours. To provide the required moisture, phosphorus and nitrogen, 200 ml of distilled sterilized water with 2 g of NH_4NO_3 and 0.2 g of K_2HPO_4 were added into the microcosms. For control microcosms only sterile distilled water was used. All microcosms were prepared in uniform plastic containers (23 × 17 × 15 cm) and equal numbers of holes (0.5 cm in diameter) were perforated on their lids to facilitate the air flow, and were incubated at room temperature (25°C). Each week the soil microcosms were aerated and mixed thoroughly using a spatula and their weight loss were evaluated and compensated by deionized sterile water. The total microbial count of the samples during the experiments was determined by standard plate count (SPC)

method on R2A-agar. According to the salinity of each soil microcosm, NaCl was added to the culture media and the colonies were counted using standard colony counter (SC6 Plus, Stuart) after one-week incubation at 25°C.

2.3. DNA extraction

After one month of microcosms test, 1 gr soil sample was taken from each microcosm and total microbial DNA was extracted by NucleoSpin® Microbial DNA isolation kit (Macherey-Nagel) according to instructions given by the manufacturer. The quality and concentration of the extracted DNA were analyzed by Nano-Drop UV–Vis spectrophotometer as previously reported. The structural integrity of the DNA was studied by 1% agarose gel electrophoresis. The DNA samples were preserved at -80°C until further analysis.

2.4. Soil bacterial diversity analysis

The soil DNA samples were used to determine the bacterial diversity by 16S rRNA amplicon sequencing. Single-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Quality filtering on the raw reads were performed under specific filtering conditions to obtain the high-quality clean reads by QIIME (V1.7.0). The reads were compared with the reference database (Gold database) using UCHIME algorithm (UCHIME Algorithm) to obtain the effective reads.

2.4. 1. Illumina sequencing

16S rRNA of distinct regions (16S rRNA V3-V4) were amplified using specific primer (515F-806R). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Finally, the libraries generated with NEBNext® UltraTM DNA Library Prep Kit for Illumina and quantified via Qubit and Q-PCR and then sequenced by Illumina HiSeq 2500 platform with 250 bp paired-end reads. All sequence data were submitted to NCBI under BioProject accession number PRJNA681110.

2.4.2. Statistical and bioinformatics analysis

In order to compute Alpha diversity, the complexity of species diversity was analyzed via several indexes, including Chao 1, ACE, Shannon and Simpson using QIIME (V. 1.7.0) and displayed by R software (V. 2.15.3). In addition, beta diversity was analyzed on both weighted and unweighted UniFrac by QIIME. PCoA (Principal coordinate analysis) was also used to identify the differences between the samples based on the beta diversity distance matrix.

2.5. Chlorpyrifos and deltamethrin extraction and analysis

The residual concentration of chlorpyrifos and deltamethrin in the samples was measured by gas chromatography/mass spectrometry (GC/MS). Each 20 g of the soil sample was mixed with 25 mL acetone and stirred at 150 rpm for 2 h. Then, the samples were washed with acetone three times, and the extracts collected and filtered through a Buchner funnel. The remained organic filtrates were concentrated

under gentle nitrogen flow, dried over anhydrous sodium sulfate and kept at 4 ^{o}C until analysis (Wong et al. 2010). GC/MS analysis was performed using Agilent 7890A chromatogram (capillary column, 30 m length, 0.250 mm inner diameter and 25 µm film thickness) and Mass selective detector (5975C VL MSD with Triple-Axis). The carrier gas was Helium at 99.99% purity under 1 mL/min flow rate.

3. Results And Discussion

3.1. Impacts of the pesticides on soil bacterial flora

To analyze the species diversity of the soil samples, all Effective Reads were grouped by 97% DNA sequence similarity into the same operational taxonomic units (OTUs). By comparing the number of OTUs and detected species (Fig. 1), it can be concluded that soil microbial diversity was significantly affected by chlorpyrifos and deltamethrin contaminants (Fig. S1, Supplementary Materials). Both of the pesticides showed considerable negative impacts on soil microbial diversity but chlorpyrifos had stronger effect on reducing OTU and observed species numbers in all soil samples regardless of the soil microbial activity and salinity. The effect of chlorpyrifos on diversity of soil microbial community was analyzed using high-density DNA microarray (PhyloChip) and demonstrated that chlorpyrifos is able to destroy a vast number of soil microorganisms (Storck et al. 2018).

Comparing the number of observed species between different salinity soils reveals a direct effect of soil salinity on microbial diversity in no-pesticide control microcosms, as the number of species decreased from 1131 to 609 by increasing salinity from 0 to 4 percent (Fig. 1). Using a 16S rRNA Miseq-sequencing study phylogenetic compositions, diversity and structure of soil microbial communities under different salinity conditions and shown that soil prokaryotic diversity decreased with salinity (Zheng et al. 2017). In another study, impacts of salinity on the soil microbial community along a natural salinity gradient was investigated in Gurbantunggut Desert, Northwestern China. The findings revealed that the microbial diversity linearly decreased in higher salinities, and community dissimilarity significantly increased with salinity differences (Zhang, Shi, et al. 2019). The addition of the pesticides to the soil microcosms (C and D microcosms) drastically decreased the soil bacterial diversity which is exhibited in lower OTU numbers in pesticide contaminated microcosms in comparison to the correspondent uncontaminated soil (Fig. 1). The higher OTU count for the 1% salinity microcosms could be due to higher biological activity of the soil (Jiang et al. 2006).

3.2. Soil microbial community structure

The soil bacterial community analysis has been extensively used to evaluate the environmental side effects of common chemicals which are applied to soil such as pesticide in agricultural industry. As indicated in Fig. 2, exposing the soil samples to deltamethrin (DM) and chlorpyrifos (CP), considerably changed the community at the genus level by relative abundance. Therefore, *Sphingomonas* which was the dominant genus in the control samples (group S), was dramatically reduced to about 0.6% and 0.8% in relative abundance by addition of CP and DM, respectively. While, the abundance of genus *Bacillus* with about 3.3% in the control samples, significantly increased to more than 37% in the group C.

From the results it may be concluded that genus *Bacillus* is one of the main CP degraders in the studied soil samples. The current findings is in consistent with Anwar et al. who found that *Bacillus pumilus* strain was able to degrade CP within 10 d (Anwar et al. 2009). There are lots of studies on the biodegradation of CP by genus *Bacillus* which prove the results (Aceves-Diez, Estrada-Castañeda, and Castañeda-Sandoval 2015; Zhu, Zhao, and Ruan 2019; Oladipo, Burt, and Maboeta 2019; Chandrashekar et al. 2017). However, studying the sample C0 (chlorpyrifos-contaminated non-saline soil) revealed that in non-saline soil sample, relative abundance of *Bacillus* surprisingly increased to more than 97%, while the other genera were about to completely disappear.

In comparison to C0, the sample D0 was rich in *Pseudomonas* genus (60% abundance) and it should be due to the deltamethrin natural attenuation ability of this genus (Yang et al. 2018).

The results, as shown in Fig. 2, indicated that the microbial diversity of the non-saline soil is more affected by the pesticide amendment in comparison to other saline soils. This phenomena could be somehow related to the adaptation of the halophiles to the extreme environment (Sato 1987). According to the results, *Paenibacillus* (D2), *Bacillus* (C4), *Paeniclostridium* (D1, C2) and *Lachnospiraceae* (C1) were the dominant genera which showed 77%, 50%, 41% and 39% relative abundance, respectively.

In this context, investigating the microbial biodiversity at phylum level revealed that the Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria were the dominant phyla which were selected by DM and CP chemicals (Fig. 3).

The study has confirmed the findings of Li et al. (2017) who reported that Firmicutes and Proteobacteria were the deltamethrin-resistant bacteria (Li et al. 2017). This resistance to DM is due to hydrolysis of ester bond by esterase/carboxyl esterase enzyme which provide DM as a carbon and nitrogen source for microbial growth and activity (Bhatt, Huang, et al. 2019; Bhatt, Bhatt, et al. 2019). Fig. S2 (supplementary materials) presents further information on the soil samples microbial diversity.

3.2.1. Alpha diversity analysis

To measure the number of different species (as the species richness) alpha diversity analysis was applied. Comparing the number of observed species, the highest species was observed in the sample S0 (non-saline, clean soil). While, the lowest observed species was detected in the presence of DM (at 1% salinity) and CP (at 4% salinity).

To determine the species richness and evenness, Shannon, Simpson, Chao1 and ACE indexes were used. According to the results shown in Table 2, the maximum indexes were observed in the group S samples (uncontaminated soils). It indicates that the microorganisms were distributed in all the samples and higher species with the same abundance level exist in the group S. Vast number of studies have been performed on the toxicological impacts of pesticide contaminants, but none considered the salinity as an effective factor on the soil microbial community composition and pesticide remediation (Wang et al. 2019; Wahla et al. 2019; Essa et al. 2019; Regar et al. 2019).

Shannon index was significantly affected by chlorpyrifos in 4% salinity microcosm. This pattern was also observed in other microcosms indicating lower richness in contaminated microcosms. The differences between CP and DM microcosms are highlighted in Table 2. The Chao1 and ACE indexes showed that species richness in deltamethrin amended microcosms was significantly lower than the richness of the chlorpyrifos-contaminated soils in 1 and 2% salinity microcosms but in non-saline and 4% salinity microcosms deltamethrin amendment increases the richness.

Overall, the results suggest that Shannon and Simpson indexes are more sensitive to the microbial community evenness, while, Chao1 and ACE indexes are changed by the community abundance which were in consistent with Zhang et al. (Zhang, Wang, et al. 2019).

3.2.2. Beta diversity Analysis

The differences between the microbial communities based on their composition was studied by principal coordinates analysis (PCoA). Beta diversity analysis at 36.24% and 25.6% of total variation for PC1 and PC2, respectively (Fig. 4.a) proved the results obtained from alpha diversity analysis which showed that the higher diversity richness was dedicated to the group S (non-contaminated soil). The most exciting part of the results was the effect of soil salinity which changed the soil microbial community resistance to the chemical contaminants.

As illustrated in Fig. 4a, the samples C1 and D4 were separated from the other samples which indicate their higher richness than the other contaminated soil samples. The other quantitative results also showed acceptable consistency with alpha diversity results. Moreover, studying the qualitative results by unweighted UniFrac PCoA revealed that the diversity composition of the samples S1 and S0 in 1% and 0% salinity were relatively same. While, the highest detrimental effects of deltamethrin and chlorpyrifos were observed in D0 and C0 microcosms, respectively which is shown in Fig. 4.b. The results verify the data presented in Fig. 3 on the samples relative abundances. Further information on the sample's beta diversity is provided in Fig. S3 (supplementary materials).

3.3. Effect of chlorpyrifos and deltamethrin on soil culturable bacterial flora

Chlorpyrifos and deltamethrin showed considerable impacts on the soil culturable microorganisms. The standard plate count (SPC) on R2A-agar showed that the microbial population was significantly reduced by addition of chemical contaminants. The microbial count data is in consistent with OTU analysis (Fig. 1a) indicating lower OTUs in pesticide amended microcosms in all salinities. The microbial count of the soil microcosms was raised again by adaptation of the microbial communities to the conditions within 50 d (Fig. 5). This phenomena was due to the gradual selection for the chlorpyrifos and deltamethrin degrading bacteria and contaminant elimination which was consumed for the microbial growth and activity (Kapta et al. 2020). Comparing the detrimental effects of the pesticides on soil microbial activity shows that both deltamethrin than chlorpyrifos reduced the number of active bacteria and the microbial count followed almost the same pattern in all soil microcosms. Figure 5 indicates that

after around 150 days of the pesticides contamination, the microbial population was started to recover which might be due to complete degradation of the pesticides and their metabolites in the media.

3.4. Chlorpyrifos and deltamethrin natural attenuation

To monitor the pesticide natural attenuation in soil microcosms the remained chlorpyrifos and deltamethrin concentrations in the samples were measured by gas chromatography-mass spectrometry (GC/MS). Figure 6 illustrates the CP and DM natural attenuation in the soil media by microbial activities. The results showed the higher contaminant natural attenuation of CP in slightly saline soils with 1% and 2% saline soils and faster removal of DM in 1% saline soil in comparison to non-saline control microcosm. As indicated in Fig. 6, it took more than 50 days for complete DM and CP natural removal from non-saline soil microcosms, while, only 20 and 25 d was needed for their natural degradation in 1% salinity, respectively.

However, increasing the soil salinity from 1–4% resulted in dramatic decrease in the natural attenuation efficiency. As shown in Fig. 6, the optimum condition for the natural attenuation was 1% salinity of the soil. By consuming the contaminants more than 40% of them were removed within first 10 d. After that, the natural attenuation slope was dramatically reduced and the remaining pesticides were slowly removed till end of the test. Previous studies showed that by increasing salinity, the microbial composition of the soil changed to halotolerant- bacteria and lots of species which was able to remediate DM and CP were disappeared (Storck et al. 2018).

Hence, CP and DM natural attenuation is a time consuming process that takes more than two months for complete remediation (Fig. 6), accelerated-remediation of deltamethrin and chlorpyrifos from soil would be helpful in achieving faster contaminant removal and lower environmental impacts (Budarz et al. 2019; Aswathi, Pandey, and Sukumaran 2019; Fatima, Tallat, and Singh 2019). There is several physical, chemical and biological approaches for xenobiotic removal from soil, in which, biological methods (bioremediation) are considered more economical and clean processes (Dar, Kaushik, and Chiu 2020). For further investigation, application of biostimulation and bioaugmentation methods is suggested for contaminated sites bioremediation.

4. Conclusion

Improper pesticides application can causes serious damages to the environment. In this study, the impact of organophosphorus (chlorpyrifos) and pyrethroid (deltamethrin) compounds on the soil microbial composition were investigated. The results proved the significant impact of soil physiochemical condition (salinity) on the microbial resistance to contaminants. The highest species number were observed in the non-saline clean soil sample and addition of CP and DM to the samples resulted in significant OTU reduction in all salinities. Furthermore, the genera *Paenibacillus*, *Bacillus*, *Paeniclostridium* and *Lachnospiraceae* were identified as the genera with highest resistance to deltamethrin and chlorpyrifos toxic compounds and possible candidates for contaminant removal from the soil microcosms. According to the results, Shannon and Simpson indexes were more sensitive to the

microbial community evenness, while, Chao1 and ACE indexes were changed by the community abundance. Additionally, principal coordinates analysis (PCoA) results were in consistent with those from alpha diversity analysis. Investigating chlorpyrifos and deltamethrin natural attenuation indicated that for the contaminants complete removal from soil samples at least 60 days is needed. Meanwhile, slight salinity (about 1%) of the soil reduced the natural remediation time but higher salinities (4%) decreased the removal rate. Therefore, the soil natural characteristics play a key role in the microbial diversity resistance to the pesticides contamination. These results could be helpful in strategy selection and monitoring of the environmental remediation approaches however further investigation on application of biostimulation and bioaugmentation techniques is needed.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets (raw nucleotide sequences) generated during the current study are available from NCBI under BioProject accession number PRJNA681110.

Competing interests

The authors declare that they have no competing interests related to the publication of this manuscript.

Funding

This study has been supported by Research Institute of Petroleum Industry (RIPI) under the grant number 33481271.

Author's contributions

Conceptualization [Mahmoud Shavandi]; Funding acquisition [Mahmoud Shavandi],[Ebrahim Alaie]; Investigation [Safoura Hashemi Jokar]; Methodology [Safoura Hashemi Jokar], [Azam Haddadi]; Data Analysis [Mahmoud Shavandi]; Data Validation [Azam Haddadi],Writing-original draft [Safoura Hashemi Jokar],Writing-review & editing [Mahmoud Shavandi], [Ebrahim Alaie], [Azam Haddadi].

Acknowledgment

The authors are grateful to Mr Sina Seyedpour for his contribution in soil sampling and analysis. The authors would like to thank Dr Hojatollah Kazemi for his assistance in GC analysis.

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Tables

Table 1. Physical and chemical properties of the studied soils

Sample	Sand	Silt	N	EC	pH	P	K	Fe	Zn	Mn	Cu
	(%)			($Ds.m^{-1}$)		(mg/kg)					
0	32	46	0.04	1.78	8.2	4.2	295	3.18	0.75	4.5	0.82
1	34	40	0.09	8.92	7.9	34.8	710	2.02	1.41	6.94	2.31
2	31	52	0.12	38.9	7.9	9.5	980	2.47	1.4	6.33	2.43
3	29	51	0.08	64.8	8	19.2	960	3.53	0.93	5.93	2.31

Table 2. Alpha diversity indexes. S: Control soil; D: Deltamethrin microcosm; C: Chlorpyrifos microcosm. The numbers indicate the salinity percent of the soils.

Sample	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage
C1	562	5.157	0.858	617.583	653.266	0.995
D1	135	2.884	0.792	230.577	275.827	0.997
C2	258	2.586	0.72	420.303	383.838	0.995
D2	169	2.709	0.798	242.357	294.449	0.996
C3	561	4.805	0.744	585.5	583.416	0.998
D3	841	7.142	0.945	885.5	880.613	0.996
C0	147	0.463	0.084	232.405	323.816	0.996
D0	365	3.441	0.806	407.714	432.674	0.996
S1	1131	8.321	0.992	1272.135	1260.107	0.991
S2	860	6.351	0.964	1543.791	1037.026	0.989
S3	609	6.494	0.972	700.333	717.671	0.994
S0	1331	8.069	0.986	1517.344	1505.847	0.988

Figures

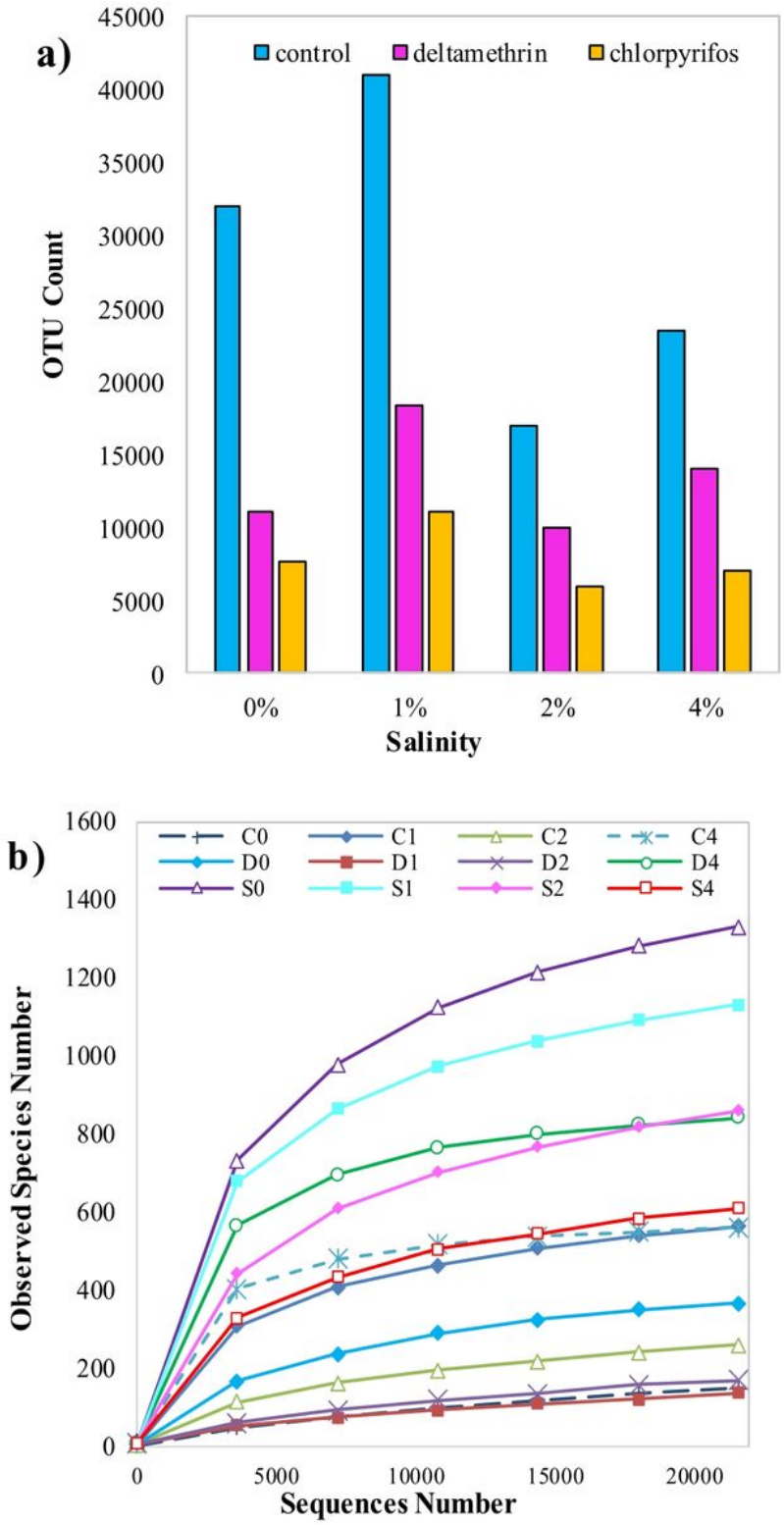

Figure 1

(a) The number of OTUs detected and (b) observes species number in the soil microcosms. S: Control soil; D: Deltamethrin microcosm; C: Chlorpyrifos microcosm. The numbers indicate the salinity percent of the soils.

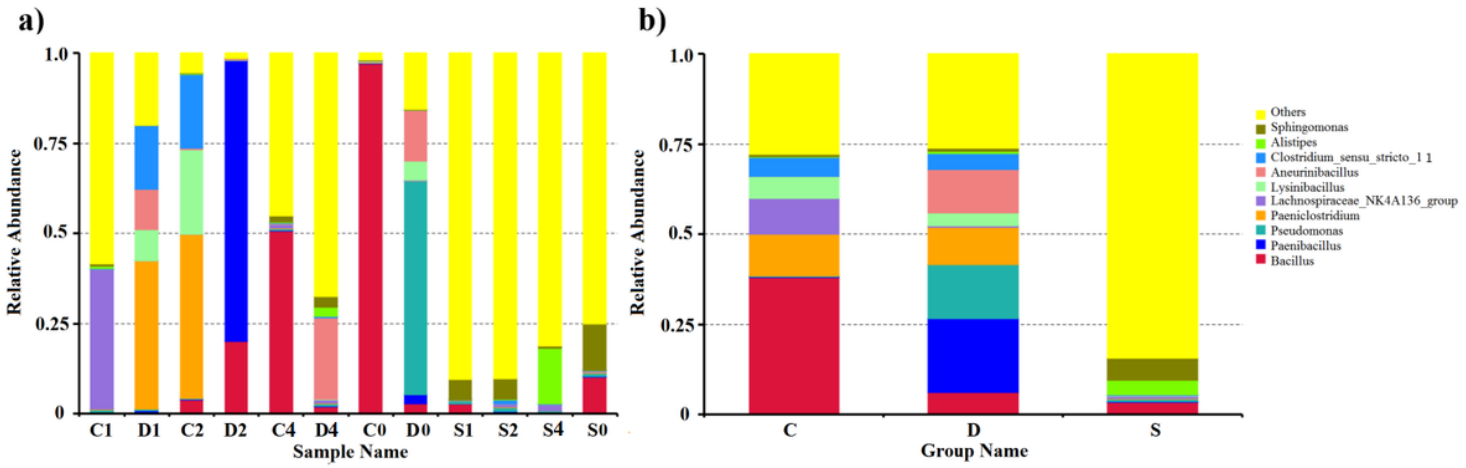

Figure 2

Species relative abundance layout at genus level based on (a) sample name and (b) group name. S: Control soil; D: Deltamethrin microcosm; C: Chlorpyrifos microcosm. The numbers indicate the salinity percent of the soils.

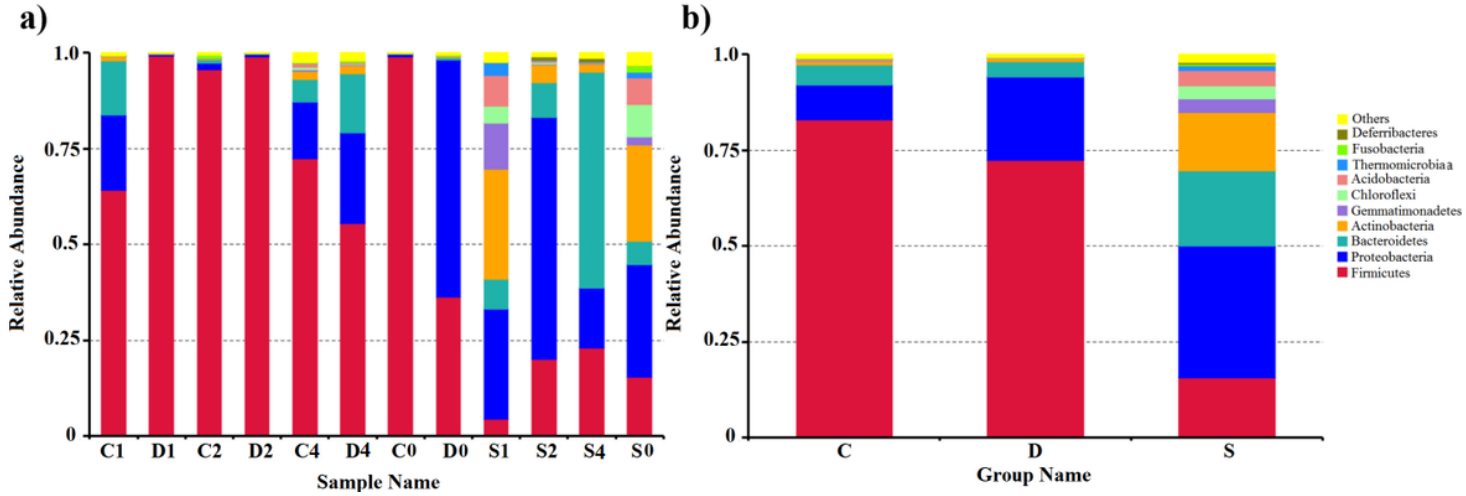

Figure 3

Species relative abundance layout at phylum level based on (a) sample name and (b) group name. S: Control soil; D: Deltamethrin microcosm; C: Chlorpyrifos microcosm. The numbers indicate the salinity percent of the soils.

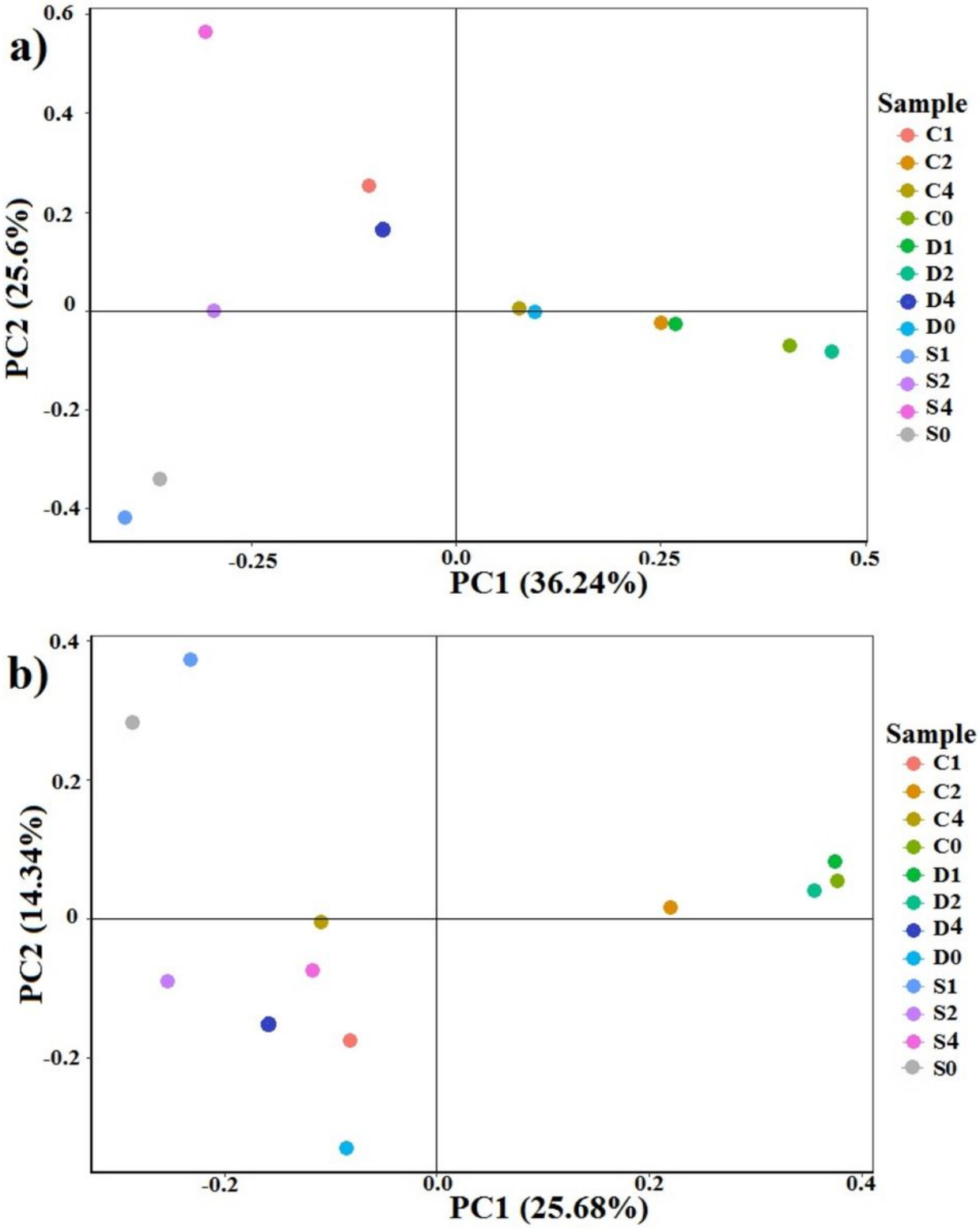

Figure 4

Two dimensional principal coordinates analysis (PCoA) of the samples. (a) Weighted UniFrac and (b) Unweighted UniFrac PCoA. S: Control soil; D: Deltamethrin microcosm; C: Chlorpyrifos microcosm. The numbers indicate the salinity percent of the soils.

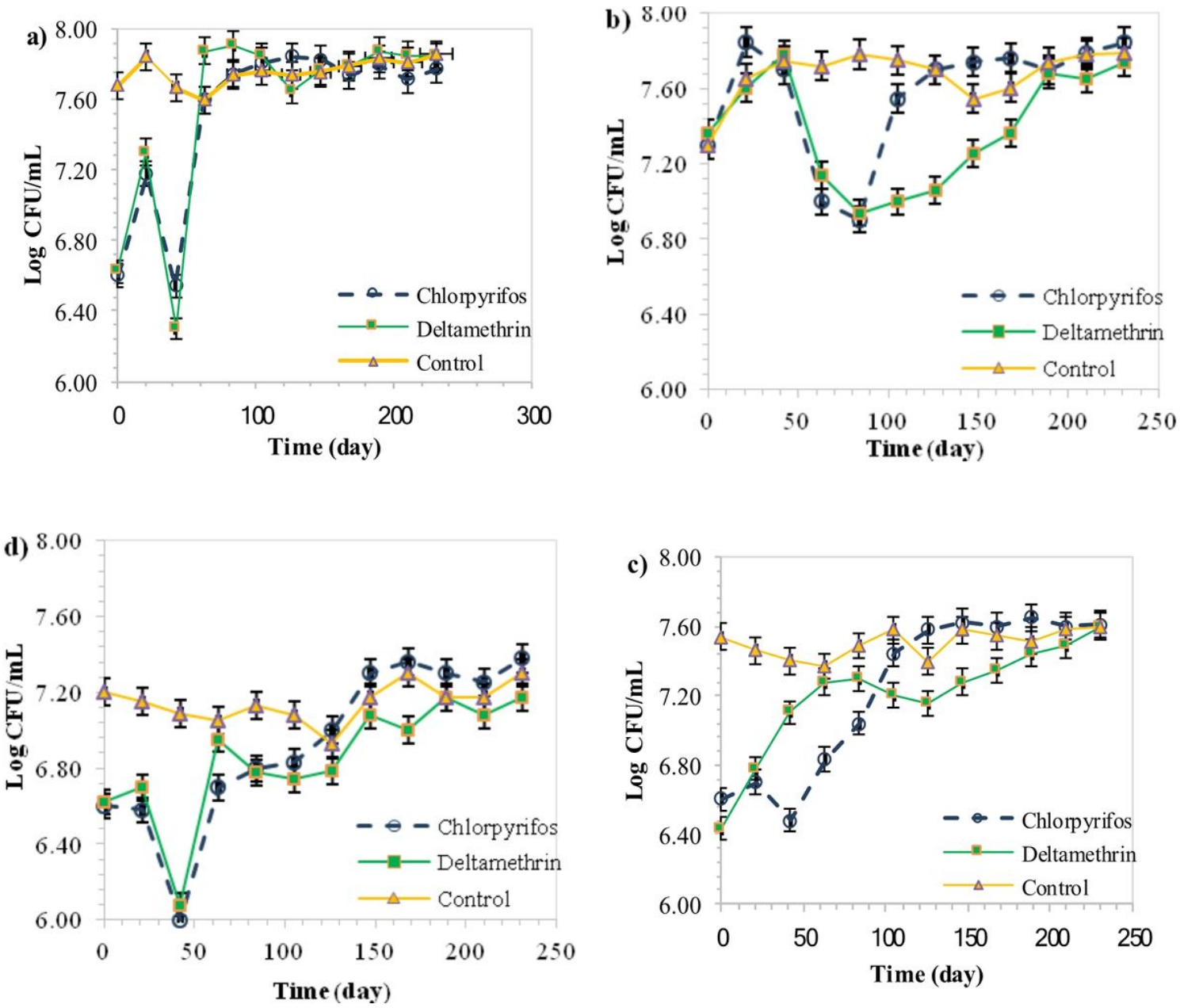

Figure 5

Dynamics of culturable heterotrophic bacterial count during remediation of deltamethrin and chlorpyrifos in a) 0%, b) 1%, C) 2% and d) 4% salinity soil microcosms.

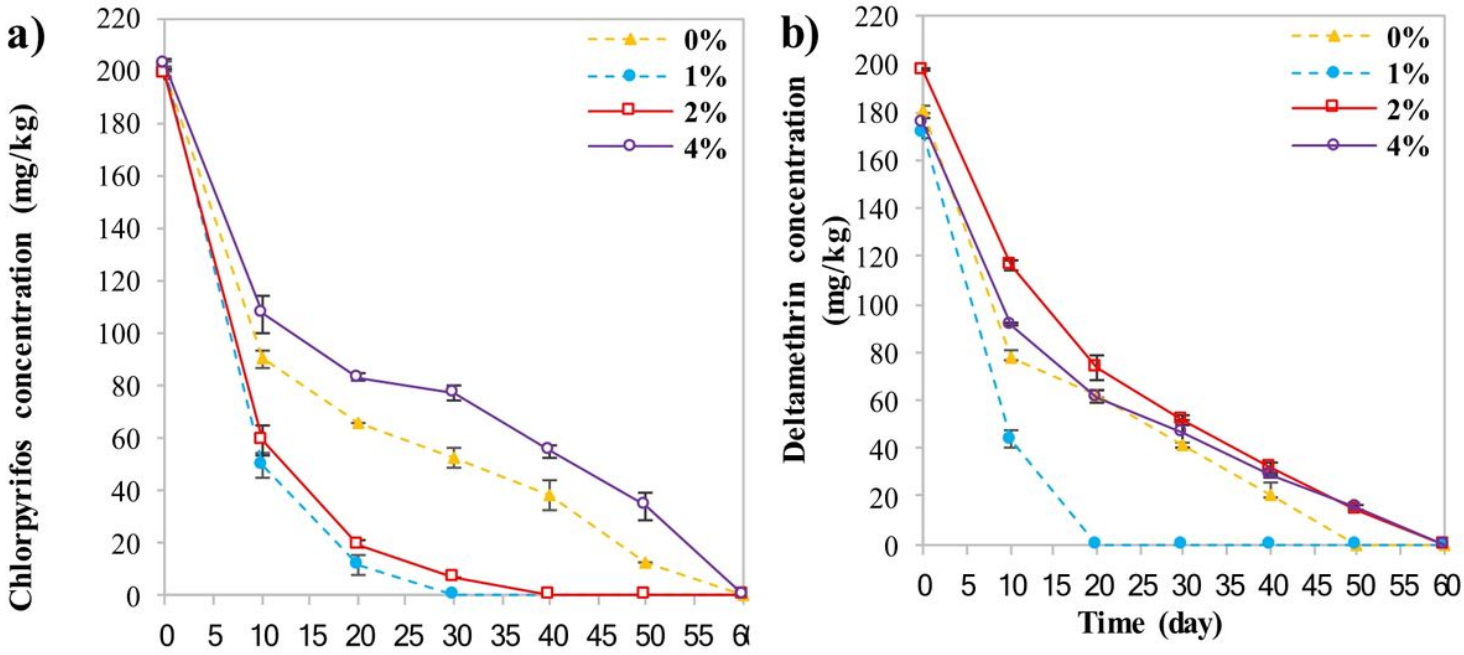

Figure 6

Time course of natural attenuation of (a) Chlorpyrifos and (b) Deltamethrin in the soil microcosms with different salinities.

Supplementary Files

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