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Effect of Pentachlorophenol Pollution Towards Microalgae and Microbial Activities in Soil from a Former Timber Processing Facility

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Pentachlorophenol (PCP), a major industrial chemical, has been extensively used throughout the world as a pesticide and general biocide in agriculture and industry. The worldwide production of PCP was estimated to be 5 x 10^7 kg by 1983 (Crosby et al. 1981). The major use of PCP (about 80%) was as a timber preservative and it has been estimated that there were over 500 wood preserving operations in the United States utilizing about 36 million kg PCP (Cirelli 1978). As a consequence of high usage and persistence, PCP is still found as a major global pollutant in the soils around timber treatment facilities. PCP has been listed as a priority pollutant by the US Environmental Protection Agency (Keith and Telliard 1979). Given the toxicity of PCP and the global distribution of PCP contamination it is important to examine the effects of the compound on soil microorganisms and essential soil functions.

The effects of PCP on aquatic algae have been studied but no information has been published on the effects of PCP on indigenous algal populations in soil. Microalgae are ubiquitous and form an important component of the soil ecosystem comprising a significant amount of microbial biomass in the ‘soil (McCann and Cullimore 1979). These organisms are involved in maintaining soil fertility as well as oxygen production (Bold and Wynne 1978). In addition, soil enzymes released by a wide variety of biota play an important role in soil organic matter degradation and nutrient cycling (Tu 1980). Therefore, any interference of PCP pollution with normal activities of microalgae and soil enzymes could result in potentially serious consequences on the overall functioning of the ecosystem. Since algae are sensitive to pollution, any changes in their composition may be useful as a bioindicator of pollution. Moreover much of the experimental work on the effects of PCP on soil microflora have been conducted by spiking the soil in the laboratory, while little data are available under field conditions. The present study was therefore aimed at evaluating the impact of PCP pollution towards the soil microflora with special reference to microalgae and on activities of three enzymes (dehydrogenase, urease, and nitrate reductase) in polluted soil and the suitability of changes in microalgal population as biological indicators of pollution.

MATERIALS AND METHODS

The study was conducted on soil from a former timber processing facility, north of

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Adelaide city, South Australia. Soil samples from depths of O-6 cm and 6-12 cm were collected using a soil auger. Five sub samples were taken at random from each depth at each sample point. Sub samples were combined, passed through a 2-mm screen and assayed. Uncontaminated control soils were collected from the same site approx. 10 m away from the contaminated site. Soil texture was determined by the hydrometer method (Day 1965) while total carbon and nitrogen were determined by the oxidative combustion method using a LECO CN 2000 Analyser. Soil pH was determined using 1:4 ratio of soil:distilled water. Microbial biomass nitrogen was estimated by the rehydration method and biomass carbon determined using a C:N ratio of 8 (Sikora et al. 1994). PCP content of the soil samples were analysed by gas chromatography after extracting with dichloromethane (Yu and Ward 1996) but without methylation. Recovery of PCP from spiked soils using this method was >90 %. Microalgal populations in the soil were estimated by most-probable number (MPN) method and algae were identified to the genus level (Muralikrishna and Venkateswarlu 1984; Megharaj et al. 1986). The populations of bacteria and fungi were enumerated as colony forming units (cfu) from lo-fold serial dilutions of the soils plated out in triplicate on agar plates and colonies counted after 5 days of incubation at 28°C. The bacterial population was estimated by using LB agar medium, and fungi with Martin's Rose Bengal agar medium.

Dehydrogenase activity in the soil was measured by incubating the soil at 37°C for 24 h with 2,3,5-triphenyltetrazolium chloride for the production of 2,3,5-triphenyltetrazolium formazan (Casida et al. 1964). The activity of soil urease was determined according to Speir et al. (1984). Nitrate reductase activity was determined as described by Abdelmagid and Tabatabai (1987). For all the assays controls were included without the substrate. All the assays were performed in triplicate and the data represent the average of triplicate values. The data were subjected to analysis of variance and means were compared by Duncan's new multiple range test.

RESULTS AND DISCUSSION

The physico-chemical properties of the polluted and unpolluted soils are presented in Table 1. These soils are predominantly sandy with a tendancy towards an increase in total carbon content in the polluted soils. There was no difference in pH among polluted and nonpolluted soils . Depending on the PCP content, polluted soils were classified as low (< 10 mg PCP kg^{-1} soil) and highly polluted soils (>800 mg PCP kg^{-1} soil). As the soil properties are similar we can reasonably assume that the microbial properties should be similar and any changes in populations and communities are due to the effects of PCP pollution.

We compared the microbiological characteristics of the soils polluted with PCP from the former timber processing facility with the unpolluted soils using standard microbiological methods. Populations of microorganisms (microalgae, bacteria, and fungi), microbial biomass carbon, and activity of their biochemical processes were determined. These parameters are useful in providing fundamentally different information about the properties of the soils and can serve as soil quality indicators. However, we put special emphasis on microalgae (eukaryotes) and cyanobacteria (prokaryotes) because of their fundamentally different cellular

Table 1. Soil characteristics and pentachlorophenol (PCP) contamination level

Soil	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Total organic carbon (%)	Total nitrogen (%)	pH	PCP (mg kg^{1})
Uncontaminated	0-6	80.0	8.7	11.3	1.51	0.134	6.6	0
	6-12	85.0	3.7	11.3	0.41	0.035	6.8	0
Low Contamination	0-6	79.0	8.5	12.5	2.65	0.122	6.9	9
	6-12	79.7	9.0	11.3	2.01	0.086	7.1	7
High Contamination	0-12	85.4	5.8	8.8	3.08	0.120	7.0	830

Soil texture and chemical characteristics were made using composite samples (at least 5 sub samples) and PCP levels estimated from composite samples in duplicate (S.D < ± 10 %).

organization and lack of knowledge on their ability to resist pollution. Viable count estimates (MPN), along with their 95 % confidence limits of algal population in the polluted and unpolluted soils are presented in Table 2. In general, the algae are more abundant in the top 0-6 cm layer of the soil. Further down the soil profile, the species diversity and abundance drops sharply. Therefore the reduction in the population size of algae in 6-12 cm layer of the unpolluted soil, compared to the top 0-6 cms are as expected. A large reduction in the population size of algae was observed in the low polluted soil (7-9 mg PCP kg^{-1} soil). Thus, there was a 13 and over 3 fold decrease in the 0-6 and 6-12 cm layers respectively, of lower level PCP polluted soils compared to the unpolluted control soil. The algae were completely eliminated in the highly polluted soil.

Table 2. Algal populations (most probable number, x $10^3 g^{-1}$ soil) in PCP-contaminated soil

Contamination level	Depth (cm)	MPN x 10^3	95 % fiducial limits	
			Upper	Lower
Uncontaminated	0-6	153.7	282.2	83.7
	6-12	15.9	29.2	8.7
Low	0-6	11.7	21.6	6.4
	6-12	4.6	8.4	< 4.1*
High	0-12	0	0	0

*Calculation of the exact lower fiducial limit (at 95%) was not feasible as only 4 culture tubes were scored positive for algae out of all 30 tubes.

Table 3. Qualitative occurrence of microalgae and cyanobacteria in soil

Organism	Level of pollution and depth (cm) of soil				
	Unpolluted		Low		High
	0-6	6-12	0-6	6-12	0-12
Chlamydomonas sp.	+	+	-	-	-
Chlorella sp.	++	+	+	+	-
Chlorococcum sp.	++	-	+	-	-
Scenedesmus sp.	++	+	+	+	-
Anaebaena sp.	++	+	-	-	-
Anabaena sp.	++	+	-	-	-
Nostoc sp.	++	++	+	-	-
Nostoc sp.	+	+	-	-	-
Nostoc sp.	+	+	-	-	-

-, absent; +, common; ++, abundant

Of particular interest in the present study was the observed change in the species composition of algae in the polluted soil (Table 3). The predominant algae in the unpolluted soil belonged to 4 species of Chlorophyceae and five of Cyanophyceae. The most common and consistently occurring forms were *Chlamydomonas* sp., *Chlorella* sp., *Chlorococcum* sp., *Scenedesmus* sp., two species of *Anabaena,* and three species of *Nostoc.* The majority of the cyanobacteria that were present in the unpolluted soil were absent in the polluted soil while 3 out of 4 species of green unicellular algae were recovered in the polluted soil. Application of PCP to an uncontaminated, but a different soil (pH, 6.1; sand,66%; clay, 3.9%), also resulted in a similar decrease in the populations of microalgae followed by alteration in the species composition (unpublished data). A similar trend has been noticed in soils treated with organophosphorous pesticides (monocrotophos and quinalphos) (Megharaj et al. 1986, 1988). A decrease in populations of diatoms, cyanobacteria and filamentous forms in an alluvial clay-loam soil under flooded, water-logged conditions and contaminated by PCP (0.8-2.0 kg ha^{-1}) has been reported (Ishizawa and Matsuguchi 1966). Changes in the species composition of marine phytoplankton communities by organochlorines, such as polychlorinated biphenyls (PCBs) and l,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), have also been reported (Mosser et al. 1972).

Unpolluted samples contained an average of 28 x 10^7 cfu g^{-1} soil of bacteria and 19 x 10^4 cfu g^{-1} soil of fungi by plate counts in the top 6 cm layer, while the lower layer contained 0.64 x 10^7 cfu g^{-1} soil of bacteria and 23 x 10^4 cfu g^{-1} soil of fungi . Low-level contaminated soil contained 2.2 x 10^7 cfu g^{-1} soil of bacteria and 36 x 10^4 cfu g^{-1} soil of fungi in the top layer, whereas the lower layer contained 1.2 x 10^7 cfu g^{-1} soil of bacteria and 29 x 10^4 cfu g^{-1} soil of fungi. In the highly contaminated soil, fungi were totally inhibited while bacterial counts corresponded to the counts in the low-level contaminated soil (data not shown). Results of plate counts indicate over a 12-fold decrease in bacteria in the low-level contaminated soil compared to the unpolluted soil in the top layer. No change in bacterial plate counts was observed between the high and low polluted samples indicating no correlation between the concentration of PCP and bacterial counts.

Microbial biomass carbon content decreased with increasing soil PCP concentration, being 89% less in the highly polluted soil compared to unpolluted soil (Table 4). The measurement of intracellular dehydrogenase activity in soil is a common method of estimating soil microbiological activity (Casida et al. 1964; Trevors 1984) and has been recommended as a measure of the side effects of agrochemicals (Gerber et al. 1991). Urease and nitrate reductase are important enzymes in nitrogen metabolism and denitrification processes, respectively (Bremner and Mulvaney 1978; Abdelmagid and Tabatabai 1987). Among the soil enzymes tested, dehydrogenase was the most sensitive with total inhibition followed by urease and nitrate reductase accounting for 96 and 95 % inhibition, respectively, in the highly polluted soil compared to the unpolluted soil. In the low level (9 mg PCP kg^{-1}) polluted soil (O-6 cm layer), microbial biomass carbon and microbial activities were only 70-90 % of those in the unpolluted soil. The inhibitory effect of PCP on urease would lead to a decrease in the transformation of urea to NH_4^+. Inhibition of nitrate reductase prevents nitrate transformation to NH_4^+ via NO_2- that may lead to leaching of NO_3^--N through deeper soil layers.

Table 4. Impact of PCP pollution on microbiological properties of soil'

Soil	Depth (cm)	Biomass carbon	Dehydro-genase	Urease	Nitrate reductase
Unpolluted	0-6	100	100	100	100
	6-12	100	100	100	100
Low Pollution	0-6	28.6*	12.7*	23.5*	18.8*
	6-12	23.4*	96.0	74.7*	36.4*
High Pollution	0-12	11.4*	n.d	3.8*	4.8*

[1]Data expressed as percentages in relation to their respective unpolluted control samples with average deviation of the mean (n=3) less than 8 %.
*Significantly different ($p \leq 0.05$) from respective controls.
n.d, not detected.

PCP is considered to be one of the most toxic chlorophenols. The toxicity of these lipophilic xenobiotics is primarily linked to the disruption of lipid membranes (Cascorbi and Foret 1991). Also, PCP is a well known protonophoric uncoupler of energy transducing membranes such as those of mitochondria and chloroplasts (Smejtek 1987). Modification of soil microflora due to PCP has been reported (Kato et al. 1981). The inhibitory effects of PCP on microflora and their activities have also been reported (Ishizawa et al. 1961; Tam and Trevors 1981; Van Beelen and Fleuren-Kemilä, 1993). Interestingly, Sato (1983) observed a strong inhibitory effect of PCP on autotrophic processes such as nitrification, whereas heterotrophic processes such as ammonification were not greatly affected in soil percolated with glycine.

Our study supports the hypothesis that PCP pollution exerts a negative influence on the biological properties of the soil as manifested by the observed decrease in populations of different microflora and altered enzymatic activities. Thus, most of microbial activities and recycling of nutrients in soil will be affected. Furthermore, an estimation of microalgae and cyanobacteria proved to be an important criterion for detection of PCP pollution and because of their sensitivity to synthetic chemicals, alteration in algal species composition can serve as a useful bioindicator of pollution. Given the sensitivity of algae to PCP we are examining their use as ecotoxicity indicators.

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