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Pesticide waste leachate toxicity evaluation and hazard quotient derivation by *Allium* assay

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ABSTRACT

This study was envisaged to explore the impact of pesticide solid waste leachate to *Allium cepa* (common onion) bulbs. Bulbs exposed to the leachate showed hampered root growth and morphological deformities. At 15% and higher concentrations of leachate after 5 days, gall like swellings were noticed around the mitotic zone (zone of root growth). From the dose response curve, the EC_{50} values for the 5th, 10th, 15th and 20th day were calculated and the highest EC_{50} value, 24.9%, was for the 5th day while the lowest EC_{50} value, 20%, was for the 20th day. The EC_{50} decreases from 5th to the 20th day successively. The 5th day EC_{50} was used to determine the hazard quotient (HQ) of the pesticide waste leachate from the dumping site. A value of 4.49 as HQ suggests that that there is considerable risk, as any value of HQ above one (>1) is environmentally unacceptable. The response in root growth pattern, residue analysis and the HQ indicates that the leachate is toxic to rot growth of onion and steps for proper management of hazardous waste and leachate are urgently required.

Keywords: *Allium cepa*; bioassay; pesticide waste; leachate; EC_{50}; hazard quotient

INTRODUCTION

According to a 1997 market estimate, approximately 5684 million pounds of pesticide active ingredients are applied throughout the world[1] and the release of waste from all pesticide industries will be in direct proportion to the quantity of pesticide produced. Pesticides leaching from agricultural fields and dumping sites to groundwater is an environmentally relevant and highly variable process[2]. Sub-surface leaching of contaminants from landfills and waste dumps causes severe degradation of the groundwater, at many places, exceeding the WHO permissible limits in drinking water[3]. Water quality impairment due to leachate contamination can occur due to either percolation downwards or lateral movement according to the slope[4], deteriorating the surrounding water bodies. Several monitoring studies have revealed groundwater contamination by pesticides[5].

Risk from solid waste dumps/disposals and contaminated soils has been reported in many studies[6,7] and risk characterization is the estimation of the incidence and severity of the effects likelihood to occur in an environmental compartment due to actual or predicted exposure to a chemical. The hazard quotient (HQ), ratio of predicted or measured environmental concentrations (PECs) to predicted no-effect concentrations (PNECs), is used for risk characterization[8]. The present study deals with the residue analysis of the leachate of waste produced by a pesticide industry, engaged in commercial production of lindane (gamma isomer of hexachlorocyclohexane, ã-HCH), its effect on the growth of *Allium cepa* root and derivation of risk quotient associated with the pesticide waste dump.

MATERIAL AND METHODS

Pesticide Waste and Leachate

The pesticide solid waste for the preparation of the leachate was collected from the freshly deposited dumping site of a pesticide industry situated at Chinhut, Lucknow, Uttar Pradesh, India. The samples were brought to the laboratory in

airtight bags. The batch leaching test EN12457-2 proposed by the European Commission[9] (EC), was employed to prepare the leachate for the toxicity testing.

Residue analysis of leachate

Pesticide residue analysis was performed as per methodology of Singh *et al.*[10].

Test organism and growth conditions

Bulbs of common onion, *A. cepa* (diameter 15-20 mm, weight 3-4 gm), were purchased from a certified shop and were properly cleaned, after peeling out the outer scales the bulbs were left under running water for 30 minutes before using for experimental purpose. During the experiment the bulbs were maintained in a BOD incubator at a temperature of 20±2°C in darkness for proper root growth.

Test solutions

The leachate was diluted with tap water to obtain the desired test concentrations of 5.0%, 7.5%, 10%, 15%, 20%, 25%, 50% and 100%. A similar set of control was also run in parallel along with the test concentrations with tap water as growth medium.

Determination of EC_{50} value

Bulbs were placed over test-tubes filled with different test concentrations of the leachate. For each concentration three bulbs were used. Using no pregrowth of the roots, the experiment will mimic a continuous exposure that is often prevailing in the environment or in daily life. Concentrations were renewed every 24-hour and the length of root bundles, length of ten roots from each bulb, were measured on the 5th day as described by Fiskesjo[11]. The root lengths were also measured on 10th, 15th and 20th day. The lengths of the root bundles in control bulbs were also measured. Considering control root length as standard, average length of the roots of treated bulbs on the 5th, 10th, 15th and 20th day were plotted against different concentrations and EC_{50} values test were calculated by graphical interpolation[12].

Elucidation of the risk quotient

Characterization of risk by the generation of leachate from the waste site is performed by using the EC_{50} value of the leachate for *A. cepa* exposed to 5 days. PEC was determined by direct monitoring i.e., chemical residue analysis of the leachate[13] while PNEC was calculated from the lowest EC_{50} value and applying the extrapolation factor, according to Calow and Forbes[14] and Girling et.al.[15]. The HQ was derived by the use of PEC/PNEC[16].

RESULTS

Residue analysis (Table 1) of the pesticide waste leachate shows that it contains 205.50±41, 167.69±1.52, 438.01±6.22 and 87.2±1.13 µg/l of α, β, γ and δ hexachlorocyclohexane (HCH) respectively i.e., 0.898 mg/l of HCH (sum of all the isomers).

On the 5th day in 5% concentration, 98.8% growth was recorded while at 100% leachate concentration, root growth was 4.1%. On the 10th day, the growth in 5% concentration was 99.5% while at 100% concentration growth was 3.9%. On the 15th day the difference between 50% and 100% concentration was maximum (39.94%) and on 20th day this difference was 40%.

Table 1: Analysis of residues in leachate of pesticide waste collected from the dumping site

S. No.	Hexachlorocyclohexane (HCH) Isomer	Values (µg/l)
1.	α- HCH	205.5±4.10
2.	β- HCH	167.69±1.52
3.	γ- (HCH	438.01±6.22
4.	δ- HCH)	87.2±1.13

Table shows the values obtained after the analysis of the leachate of the pesticide solid waste; the various isomers of HCH are present in varying amounts and are subsequently used to derive the predicted or measured environmental concentration (PEC).

Determination of EC_{50} value

EC_{50} values of *A. cepa* root growth were 24.25%, 21.49%, 20.6% and 20% (Fig. 1) on 5th, 10th, 15th and 20th day respectively. Besides control, maximum growth was recorded in 5%, 7.5%, 10%

and 15% concentration during 5th to 10th day. However, reduction in growth was observed in these sets during 10th to 15th and 15th to 20th day. In the 100% concentration the roots ceased to grow from 8th day onwards. During the total exposure period the maximum growth (for a 5 day period) of 51.4% was recorded for the 50% concentration during 10th to 15th day. On the 5th day at 25% (H" 5th day EC_{50}) concentration, the roots showed curls and twists. On the 20th day at 20% (= 20th day EC_{50}) concentration gall like swellings were noticed around the root hair zone.

Elucidation of the hazard quotient

The PEC of 0.898 mg/l was derived from the residue analysis of the leachate while PNEC was calculated, as 0.20 mg/l. Risk from leachate generation in the form of hazard quotient was determined to be 4.49. The residue analysis of the leachate gave the PEC and the EC_{50} was used to derive the PNEC, which were later used to characterize risk.

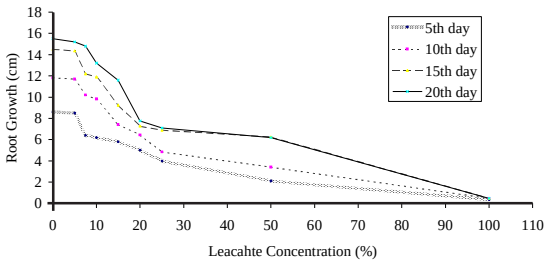

Fig. 1: Growth of *A. cepa* root on 5th day, 10th, 15th and 20th day (EC_{50}=24.25%, 21.49%, 20.6% and 20% respectively)

DISCUSSION

The EC_{50} values decreased with increase in the exposure duration. A finding by Ward *et al.*[17] 2002 in which landfill leachates were shown to have high toxicity to both *C. dubia* and *S. capricornutum* with an EC_{50} < 10% and < 15% respectively suggests that the leachate we tested in this study was moderately toxic. The abnormalities in the growth of roots were in agreement with Fiskesjo[18], according to which the growing roots tips may, after various treatments, take the shapes of hooks, spirals or tumours. Such observations may give information of specific action of a chemical[19]. The growth of *Allium* root was inhibited by the leachate in a dose-dependent manner and any inhibition of growth reflects toxic effects on metabolic processes[20]. Results of study of comparative effects of insecticides on the *Allium* and mammalian test systems indicate that the *Allium* test responds to these insecticides similar to mammalian test systems[21] and hence elicits a correlation between *Allium* root meristem cells and *in vivo* / *in vitro* mammalian test systems. These results can be used to relate the leachate toxicity to mammals; since the leachate is toxic to *Allium* (hampering the root meristem cells) it will be toxic to mammals also.

Similarly obtainment of PEC, PNEC and RCR values was accomplished by Palma, *et. al.*[22,15]. A PEC/PNEC quotient e" 1 signifies that there is risk of effects occurring and a large quotient is likely to indicate a high level of risk[14]. Thus a RCR of 4.492 indicates towards moderate to high risk.

Fisksejo[11] and Chauhan and Sunderaraman[23] have given consideration for 5th day measurement of root lengths. This 5th day concept may have arisen for the assessment of toxicity of pesticidal sprays and formulations because the pesticides are applied at a particular time and there are intervals between the successive applications. So the impact of pesticide can be evaluated after 5th day from the time of exposure. But in case of leachate toxicity from a solid waste site there is continuous leaching to the nearby soil or water body, therefore, the test was extended to 20 days. A growth period of 48 or 72 hour is suitable as a standard; after 7-8 days the differences between treated and control roots were even clearer, and thus the longer treatment preferred[19]. The EC_{50} values, for root inhibition, reported here can be used to determine appropriate leachate concentrations for genotoxicity assays.

As a conclusion it is recommended that a proper disposal of solid waste and regular monitoring of ecological effects of the leachates generated from such dumping site is essential for preservation of water quality and ecosystem health.

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