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Research Paper

Sublethal Impact of Nickel on Survival and Soil Metabloism of an Anecic Earth Worm.

Ecology

KEYWORDS : Lampito mauritii, survivility, soil metabolism, nickel fungicide

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ABSTRACT

Experiments conducted on Lampito mauritii earthworm exposed to different sublethal levels of nickel gave following results.

On exposure to sublethal doses of nickel, the adult worms survived for longer duration than immature worms. There was significant decrease in survivility of both immature and adult worms due to impact of nickel at 0.001 level of significance (ANOVA).

Significant positive correlation at 0.05 level was found between the concentration of nickel and respiratory metabolism of Lampito mauritii. At 240 hours exposure there was increase of about 18,27 and 35% in the rate of respiration on exposure to 2, 4, 6, & 8 ppm nickel over 0 ppm nickel, respectively.

INTRODUCTION:

The simplification of diversified ecosystem to simpler agricultural and lawns containing large population of one or two desired plant species, has led to acute problem of pests and damage caused to crop yield. In the simplified ecosystems, organisms grow and reach the status of pest. But in the diverse complex ecosystem this organism's population is controlled by natural prey predator relationship. This serious threat of the pests to world's mushrooming population has resulted in expenditure of time, energy and money to control pests in crop fields. The most widely used approach has been spraying of pesticides i.e. certain synthetic chemicals (Miller, 1988). Most of the effective pesticides are broad spectrum biocides which destroy beneficial species along with pests unintentionally (Lee, 1985). Research on deleterious effect of the pesticides on the terrestrial and aquatic target organisms has been done extensively (Edward & Jeffs 1973). But in contrast very few works has been done on the effect of the pesticides on non-target organisms like earthworms (Patnaik, 1994, 2002). The government policies for pesticides registration require the submission of data after assessment of toxicity of pesticide to non-target organisms. Since the earthworms play vital role in promoting soil fertility and plant growth, the use, of pesticide should involve toxicity test on earthworms.

Wide range of biocides is now available and more and more new chemical formulation and application methods are appearing. But comparatively few have been tested on earthworms (Edwards, 1980; Lee, 1985). The research publications dealing with effect of pesticides on earthworm describe percentage reduction in population and LD_{50} or LC_{50} (Edwards, 1980). A few information is available in population dynamics, change in biomass, production metabolism and energetic with exposure to various levels of pesticides. The pesticides are labelled as non-toxic if they do not instantaneously kill earthworms in certified agricultural doses. And the lethality is observed by suspension experiments in laboratories (Edwards, 1980). Very little study is available on the deleterious effect of sublethal level of pesticides on the ecology and particularly on the positive contribution of the non-target organisms like earthworm towards the soil fertility in agricultural system (Senapati et. al., 1992).

Lampito mauritii kinberg is the most common anecic earthworm distributed in varied agroclimatic zones.

In the present work an attempt has been made to study the impact of nickel on survivility and soil metabolism of the *Lampito mauritii* earthworm.

MATERIALS AND METHODS:

Nickel is used as fungicide in the tea gardens. It is utilized in the form of nickel chloride hexahydrate commonly known as liqnik or nickelchloride (NiCl2 6H2O).

Nickel and copper compounds have a strong effect on the germination of spores besides having inhibitory effect on the growth and development of fungal mycelium (Gruzdyev et. al., 1983). Nickelchloride is effective against blue mold, drowny mildew, late blight etc.

In the tea gardens the certified does of liqnik (nickelchloride) application is 265g diluted in 70 liter water per hectare . This comes to around 3.79g, l^{-1} i.e. 3790 ppm. Active ingredient of Nickel in nickelchloride is about 24.69=25% (mol. wt. of nickelchloride hexahydrate = 237.7 and atomic weight of nickel = 58.7).

Besides being used as fungicide, nickel is also consumed in production of alloys like stainless steel, alloy steels, ductile and cast irons, cupronickels and high nickel alloys. Pure nickel is also consumed in electroplating and electroforming of products such as trim and bumpers in automobiles, household appliances and plumbing fixtures. Nickel is also used in manufacture of nickel cadmium batteries for stand-by power equipment, zinc-nickel oxide storage batteries, bonding ceramics to metal and as catalyst in the preparation of edible oils and in solar energy equipment.

According to standard of tolerance limit adopted by India in 1976 and revised in 1983 general tolerance limit of nickel is 3mg.L-1 or 3ppm which could be utilize for land application of effluents (Panday and Carney, 1989).

The microcosm experiments have been conducted with nickelchloride solution containing 0,2,4 and 8ppm nickel as active ingredient. These doses have been chosen considering 3ppm nickel as the certified national tolerance limit for effluents. Experiments beyond 8ppm were not conducted because instant lethality was observed when worms were put in nickelchloride solution containing more than 8ppm nickel as active ingredient. Nickel concentration (ppm) was calculated from NickelchlorideNiCl2. 6H2O) Analar grade (BDH India make).

The field studies showed significant absence of anecic earthworm in tea garden soil. *Lampito mauritii* Kinberg is an anecic earthworm widely distributed in India including Andaman and Nicobar Islands, Laccadive and Minicoy.

Mature *Lampito mauritii* earthworm is about 95-155 mm long and 3-6 mm in diameter. It has 157-201 segments. Prostomium is epibolic, tongue closed. First dorsal pore is in 10/11 or 11/12 or 12/13 segment. Clitellum is annular. Male pores are on slightly raised porophores. Female pores are presetal. Spermathecal pores paired.

Reproduction occurs in swampy habitat (soil having moisture more than 10g %). Cocoons are oval with a hatching and a non-hatching end. Average diameter of cocoon is 3.35mm, length 4.7mm and live weight 25.61 mg. Incubation periods is about 4 months. Usually one juvenile hatches from each cocon. Juveniles become adults in about 20 months (Julka and Senapati, 1978).

Both adult and immature *Lampito mauritti* earthworms were utilized for conducting survivility experiment. The worms were exposed to 0, 2, 4 and 8 ppm nickel as nickelchloride solution for 240 hours.

Flat bottomed glass vials of about 10cm long and5cm in diameter were taken. The sides of the vials were lined with filter paper. 3ml of experimental solution was pipetted into respective vials and evaporated to dryness under low stream of air. The control vials were treated with 3ml of deionised water. After drying, 1 ml of deionised water was added to each vial to moisten the filter paper. Then each vial was sealed with a plastic film with a small ventilation hole. For each treatment 50 replicates, each consisting of one worm per vial was taken (temperature $20^0 \pm 2^0$c). Before placing the worms in the test vials their gut contents were cleaned by keeping them on moist filter paper for three hours (OECD, 1984).

Percentage of survivility with respect to time was taken into account. To draw conclusion, percentage survivility with respect to time with analysis of variance and t-tests have been done (Snedecor and Cochrane, 1967; Gupta, 1980).

Alkali absorption method (Witkamp, 1966) has been utilized to quantify respiratory metabolism. Carbondioxide evolution was measured at and has been expressed as mg of CO_2 evolved, g^{-1} live worm tissue, hr^{-1}, kg^{-1} soil.

1 kg of soil was taken in polythene which was kept undisturbed with the help of PVC pipe. The soil packet along with PVC pipe was kept inside another polythene packet. During the quantification the whole set was kept inside the glass jar and was made air tight with glass lid and grease. The soil moisture was maintained 20 ± 2g% with distilled water in case of 'O' ppm i.e. control set and by 2, 4, 8 ppm nickel as nickelchloride solution in experimental sets. 10 replicates of each set were taken. The moist soil packets were kept undisturbed for 5 days for microbial activation. To each packet i.e. 1 kg soil, 5g of earthworm was inoculated. The moisture content of each soil packet was maintained, 20 ± 2g% by addition of respective nickelchloride solution. The respiratory metabolism was maintained as 0, 48, 96, 144, 192 and 240 hours. Statistical analysis of the respiratory metabolism was made by ANOVA and t-tests (Snedecor and Cochran, 1967; Gupta, 1980).

RESULTS:
Table I and II represent percentage survivility of earthworms, *Lampito mauritii*, adults and immatures under the impact of nickel. Figure-I shows percentage survivility of earthworm *Lampito mauritii* in suspension under the impact of Nickel.

The worms could not survive beyond 96 hours of exposure in 8ppm nickel irrespective of age group. 100% mortality of adult worms occurred at 240 hours when exposed to 2 ppm nickel. At 2 ppm nickel 100% mortality of immature worms occurred at 192 hours of exposure. At 4 and 8 ppm nickel 100% mortality of adult *Lampito mauritii* worms occurred at 168 and 96 hours of exposure, respectively. 50% mortality of adult worms at 2, 4 and 8ppm nickel occurred at 137, 125 and 77 hours of exposure, respectively .Survivility after 120 hours of exposure to 2, 4 and 8ppm nickel was found to be 80%, 60% and 0%, respectively. There was significant decrease in survivility due to impact of nickel at 0.001 level of significance.100% mortality of immature earthworms occurred at 192, 144 and 96 hours of exposure in 2, 4 and 8ppm. Nickel (Table II). At 96 hours of exposure 73.33, 63.33 and 0% worms survived in 2, 4 and 8ppm nickel respectively. 50% mortality of immature worms occurred at 137, 113 and 67 hours of exposure in 2, 4 and 8ppm nickel, respectively. The adult worms survived comparatively for longer duration than the immature worms when exposed to nickel at different concentration. Analysis of variance shows significant effect of nickel on survivility of immature worms at 0.001 level of significance.

Soil Metabolism:
Table III shows the respiratory metabolic rate of *Lampito mauritii* earthworm under the impact of nickel in laboratory culture.

The rate of respiration increased with duration and treatment. At 48 hours of exposure the rate of respiration was found to be 1.35, 1.45. 2.09 and 2.41 mg of CO_2 evolved, g^{-1} live worm tissue, hr^{-1}, kg^{-1} soil at 0, 2, 4 and 8ppm nickel, respectively. At 240 hours of exposure there was increase of about 18%, 27% and 35% in rate of respiration on exposure to 2, 4 and 8 ppm nickel over 0 ppm nickel. At zero hour of exposure of worms to nickel at 0 and 2 ppm did not produce significant difference. But there was significant increase in the respiratory metabolism on exposure to nickel at 4ppm and 8ppm over 0ppm at 0.001 and 0.05 levels of significance, respectively at 0 hour of exposure. At 96 hours of exposure a significant difference in respiratory metabolism was found between 0 and 2, 4, 8ppm nickel at 0.001 level of significance. Significant positive correlation (0.05 level of significance) was found between the concentration of nickel and respiratory metabolism of earthworm and respiratory metabolism of earthworm *Lampito mauritii* at 0, 48, 96 and 144 hours of exposure. At 192 and 240 hours of exposure significant correlation between nickel concentration and respiratory metabolism was observed (significant at 0.05 level).

DISCUSSION:
Survivility of an organism depends upon the exposure of the organism to the stress, its duration and dose. The extent of toxicity determines lethality or sub-lethality. Exposure to lethal dose results in complete cessation of the biological activity. But at sublethal dose a negative impact is found on the organism which hampers the organism's positive beneficial role towards soil fertility (Achazi et. al. 1988,; Xiao Nengel et. al. 2006). In case of sublethal dose the organism stays in the system, but does not contribute towards the system as its positive beneficial role is cancelled as a result of stress impact.

Pesticides in large amount are being applied to the soil to kill and control the pests. But most of the data on metal toxicity with respect to earthworm are based on field studies (Edwards and Lofty, 1977). In the present study differential survivility capacity of *Lampito mauritii* earthworms, with variation in sublethal dose and age of worms have been worked out. Survivility of an organism indicates the dose and extent of toxicity on exposure to the stress.

Review of the impact of heavy metals has been done by Ireland (1983) indicating the accumulation of heavy metals in earthworms differs with species and type of heavy metal and various physiological factors. Impact of heavy metals on earthworms in field and laboratory has been reported Edwards, 1977; Hartenstein et. al., 1980; Malecki *et. al.*, 1982; Lee, 1985. Toxic effect of arsenic compounds copper fungicide on survivility of the earthworms has been reported by many workers (Polivka, 1951; Escritt, 1955; Raw and Lofty, 1959; van Rhee, 1967). van Rhee (1967) reported 90% decrease in earthworm population in 60 years old orchards receiving heavy metal copper continuously for 12 years. Buahin and Edwards (1963) report the negative impact of fumigants on the survivility of earthworms living in burrows in deep soil. Fox (1964) reports that TCA decreases the earthworm population. Davey (1963) has reviewed the impact of insecticides on the earthworms. Chlorinated hydrocatbons like aldrin, BHC, chlordane affect the survivility of earthworms negatively and result in decreased population (Hopkins and Kirk, 1957; Lipa, 1958; Edwards and Dennis, 1967; Long et. al., 1967; Heugens, 1969).Toxic affect of organophosphates on earthworm have also been reported byScott, 1960; van der Drift, 1963; Kelsey and Arlidge, 1968; Zhou Qi-Xing et.al., 2006.

The differential survivility of the worms with respect to age may be explained on the basis of greater ability of audlt worms to produce mucus. The mucus layer acts as a barrier for penetration of heavy metals (Fleming and Richards, 1981). In the present study it has been observed that adult worms survive for a longer duration on exposure to the different doses of nickel.

The major pathway of energy loss from the organism is represented by respiratory metabolism. More than 50% of total energy utilization of free living organisms is attributed to respiration. Determination of carbondioxide evolution i.e. soil respiration is one of the best approaches for evaluation of biological activity in terrestrial ecosystem (Lundergarh, 1927). CO2 evolution from the soil can be considered as an index of soil organisms activity (Witkamp, 1966).

Metals are associated with protein moity which are involved in physiological processes like respiration (Valley and Walker, 1970). According to Cherian and Goyer (1978) the exposure of organisms to sublethal amount of heavy metals induces synthesis of the metaloprotein, moity. This may result in enhanced respiration rate Enhancement in maintenance cost has been reported by Senapati et. al., (1992) on exposure of earthworms to malathion. The enhancement in respiration has been attributed to the increased muscular activity on exposure to 1.3 µg, g^{-1} malathion (Stenerson et. al., 1973).

Various workers have quantified the energy utilization through respiraton (Gromadska, 1962; Dash & Patra, 1977; Senapati & Dash, 1991; Patnaik and Senapati, 1996). Maintenance cost amounts to about 50% in arctic climate, to about 60% in earthworms from subtropical climate and about 70% in earthworms from tropical climate. Temperature stress results in 3 fold increase in the oxygen consumption in summer as compared to winter have been reported by Senapati and Dash (1983). Inhibition of oxygen consumption has been observed on transaction of nerve cord of earthworm due to inhibition of cholinesterase activity (Stephenson, 1930; O' Brien, 1957; Stenerson et. al., 1973). In the present study enhancement in the respiration metabolism has been observed which is resulting in the loss of more energy assimilated by the earthworms.

TABLE – I PERCENTAGE SURVIVILITY OF EARTHWORM *LAMPITO MAURITII* (ADULTS)UNDER THE IMPACT OF NICKEL

Duration of exposure to nickel solution in hour.	Percentage survivility in Different Conc. Of Nickel in ppm			
	0	2	4	8
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
12	100 ± 0	100 ± 0	100 ± 0	100 ± 0
24	100 ± 0	100 ± 0	100 ± 0	100 ± 0
36	100 ± 0	100 ± 0	100 ± 0	100 ± 0
48	100 ± 0	100 ± 0	100 ± 0	100 ± 0
60	100 ± 0	100 ± 0	100 ± 0	80 ± 0
72	100 ± 0	100 ± 0	93.33 ± 6.67	66.67 ± 6.67
84	100 ± 0	100 ± 0	93.33 ± 6.67	26.67 ± 6.67
96	100 ± 0	93.33 ± 6.67	93.33 ± 6.67	0 ± 0
108	100 ± 0	86.67 ± 6.67	80.00 ±11.55	------
120	100 ± 0	80 ± 0	60 ± 0	------
132	100 ± 0	53.33 ± 6.67	33.33 ± 6.67	------
144	100 ± 0	46.67 ± 6.67	20 ± 0	--- ---
156	100 ± 0	40 ± 0	20 ± 0	------
168	100 ± 0	40 ± 0	0 ± 0	------
180	100 ± 0	40 ± 0	-------	------
192	100 ± 0	26.67 ± 6.67	-------	------
204	100 ± 0	20 ± 0	-------	------
216	100 ± 0	13.33 ± 6.67	-------	------
228	100 ± 0	13.33 ± 6.67	-------	------
240	100 ± 0	0 ± 0	-------	------

TABLE – II PERCENTAGE SURVIVILITY OF EARTHWORM LAMPITO MAURITII (IMMATURES) UNDER THE IMPACT OF NICKEL

Duration of exposure to nickel solution in hour	Percentage survivility in Different Conc. Of Nickel in ppm			
	0	2	4	8
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
12	100 ± 0	100 ± 0	100 ± 0	100 ± 0
24	100 ± 0	100 ± 0	100 ± 0	100 ± 0
36	100 ± 0	100 ± 0	100 ± 0	100 ± 0
48	100 ± 0	100 ± 0	100 ± 0	100 ± 0
60	100 ± 0	100 ± 0	86.67 ± 6.67	83.33 ± 3.33
72	100 ± 0	100 ± 0	80.00 ± 5.77 16.67 ± 8.82	
84	100 ± 0	76.67 ± 3.33	70.00 ± 5.77	3.33 ± 3.33
96	100 ± 0	73.33 ± 3.33	63.33 ± 3.33	0 ± 0
108	100 ± 0	70.00 ± 5.77	60.00 ± 5.77	------
120	100 ± 0	63.33 ± 3.33	36.67 ± 3.33	------
132	100 ± 0	53.33 ± 3.33	16.67 ± 3.33	------
144	100 ± 0	46.67 ± 3.33	0 ± 0	------
156	100 ± 0	40 ± 5.77	-------	------
168	100 ± 0	26.67 ± 3.33	-------	------
180	100 ± 0	16.67 ± 3.33	-------	------
192	100 ± 0	0 ± 0	-------	------

TABLE – III RESPIRATORY MATABOLIC RATE OF EARTHWORM LAMPITO MAURITII UNDER THE IMPACT OF NICKEL IN LABORATORY CULTURE

Duration of exposure in hours	Rate of Respiration (mg of CO2 evolved, g-1 live worm tissue, hr1, kg-1 Soil) at Different Conc. Of Ni in ppm			
	0	2	4	8
0	1.03 ± 0.10	1.12 ± 0.16	1.64 ± 0.02	2.24 ± 0.09
48	1.35 ± 0.02	1.45 ± 0.02	2.09 ± 0.11	2.41 ± 0.09
96	1.99 ± 0.10	2.13 ± 0.15	2.62 ± 0.28	3.68 ± 0.20
144	3.47 ± 0.28	3.92 ± 0.22	4.17 ± 0.14	4.48 ± 0.13
192	3.82 ± 0.12	4.47 ± 0.24	4.48 ± 0.15	5.15 ± 0.17
240	3.87 ± 0.1	4.58 ± 0.2	4.91 ± 0.09	5.22 ± 0.27

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