Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 483 - 491

Research Article | Zoology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ECOTOXICITY OF SODIUM ARSENITE ON *EISENIA FETIDA*

PALLAVI KAUSHIK*, SONAM MEENA AND MEGHA MATHUR

Department of Zoology, The IIS University, Gurukul Marg, SFS, Mansarovar, Jaipur-302020, India.

ABSTRACT

In this study, we performed ecotoxicity tests of sodium arsenite on soil invertebrate *Eisenia fetida*. Initially, broad range of sodium arsenite (0.1, 1, 10, 100mg/ml) was tested for mortality of *E. fetida* after 24 hours in filter paper test. The narrow range doses (0-1mg/ml) were further tested to determine the EC_{50} and LD_{50}. The EC_{50} dose of sodium arsenite was considered at which 50% of earthworms exhibited rejection to the spot on filter paper in the spot rejection test. The LD_{50} of sodium arsenite resulting in mortality in 50% of *Eisenia fetida* was studied with contact filter paper bioassay (24hrs) and soil bioassay (14 days). In the range finding test, there was no mortality in control and 0.1mg/ml but 100% mortality was observed at doses of 1mg and above after 24 hours. Thus, for further study smaller doses intervals in the range between 0-1mg/ml was selected for spot rejection, contact filter paper and soil bioassay. The percent mortality in each test was computed and was subjected to regression analysis to get a linear dose response curve and estimation of EC_{50} and LD_{50}. The EC_{50} was estimated as 0.3mg/ml of sodium arsenite concentration to *Eisenia fetida*. The 24hrs- LD_{50} was estimated as 0.4mg/cm^2 in filter paper and 14 days -LD_{50} as 5mg/kg in soil bioassay. Thus it can be concluded that *Eisenia fetida* exhibit sign of toxicity in environmental soils which are containing 1mg/kg and higher doses of sodium arsenite.

KEYWORDS: Ecotoxicity, *Eisenia fetida,* sodium arsenite, bioassay, EC_{50} and LD_{50}.

PALLAVI KAUSHIK
Department of Zoology, The IIS University, Gurukul Marg,
SFS, Mansarovar, Jaipur-302020, *India.*

INTRODUCTION

Earthworm toxicity tests are currently used as a basis for international regulatory guidelines in environmental risk assessment[1]. *Eisenia fetida* are most common soil invertebrate used for vermicomposting. They represent about 60-80% of total biomass in soil[2,3]. These invertebrate have intimate contact with the soil as they do not have outer cuticle. They play an important role in recycling nutrients, maintaining the fertility as well as structure of soil and increasing aeration and drainage. Earthworms are base of many food webs and are a component of diet of birds, reptiles and mammals[4]. When present in metal contaminated soil they are known to accumulate large concentration of metals in their tissues. The accumulation of metals in tissues of earthworms not only creates toxicity but also causes transfer of metals to higher trophic levels. Earthworms are suitable for vermicompost preparation as well as ecotoxicological testing of metals like arsenic. It also show chemotaxis in presence of toxins and reject them by showing sharp turn if spot of toxic chemical is applied to its path. This chemo sensitivity can also be used to study ecotoxicity of any chemicals[5]. The ecotoxicity of chemicals and determination of its LD_{50} levels have been estimated on earthworm by various workers[4,6,7,8,9].Arsenic is known to have adverse health effects on human health like cancer[10], cardiovascular and neurological effects[11,12,13]. It is widely distributed in nature in the form of either metalloids or chemical compounds, primarily present in inorganic forms and the two predominant inorganic species are arsenate (AsV) and arsenite (AsIII). Arsenite [As(III)] is 100 times more toxic than arsenate [As(V)][14,15,16]. Arsenite ($As^{III}O^-_2$) is toxic to cells because of its ability to bind to essential sulfhydryl groups of proteins and dithiols such as glutaredoxin, disrupting intracellular oxidation- reduction homeostasis and inhibits pyruvate dehydrogenase[17,18]. Arsenic is continuously being introduced into our environment from natural and anthropogenic sources and consequently adversely affecting the biota. The toxic effects of arsenic are well known on mammals but ecotoxicological reports of arsenic on soil invertebrate are meagre. The short term acute toxicity test on *Eisenia fetida* are also desirable for soil quality evaluation because physical and chemical test does not give sufficient information about the potential effects of toxicants on soil biota. Thus, in the present study we plan to assess the toxicity of arsenite As(III) on Earthworm; *Eisenia fetida.*

MATERIALS AND METHODS

Test Organism: *Eisenia fetida*

These were purchased from the Rajasthan Agriculture University, Jaipur and bred in earthen pots with standard vermicomposting mixture with moisture content adjusted to 35% by adding distilled water and temperature maintained around 25^{o}C.Adult earthworms (at least two months old with clitellum) with an individual weight of 300 to 600 mg were used for the study. Minimum of 10 earthworms were taken for each test.

Test substrate for toxicity testing: *Sodium Arsenite*

Sodium arsenite (Hi-Media) was dissolved in distilled water to give a range of concentrations a 0.1mg, 1mg, 10mg, 100mg/ml.These doses were used for preliminary range finding test prior to a more precise screening test. The further screening tests were performed taking smaller dose interval with doses showing 0% to 100% mortality.

Acute Toxicity

Short term lethal effect of sodium arsenite was tested on *Eisenia fetida* in filter paper and artificial soil test.Lethal concentration (LC_{50}) of sodium arsenite in filter paper and soil test was determined after 24 hrs & 14 days observations, this is the toxicant concentration estimated to produce death in 50% proportion of organism.Effective concentration (EC_{50}) is the toxicant concentration estimated to cause a specified effect in 50% proportion of test organism[19].

1. ***Spot Rejection Test***

E. fetida was subjected to varying concentration (0.1-0.9 mg/ml) of sodium arsenite (Hi-Media) as a spot in its path. *Eisenia fetida* shows rejection to spot of toxicant by showing one or more random sharp turns as soon as it reaches the spot.

2. *Contact Filter paper test*

To perform this test, Flat-bottomed glass vials of 14cm in length and 7cm in diameter were used. Their sides were lined with filter paper cut to a suitable size so it does not overlap considerably. The doses of sodium arsenite were pipette out as 1ml of each solution into each vial and evaporated to dryness. The control vial was prepared with one ml of distilled water. After drying, one ml of distilled water is added to each vial to moist the filter paper. Each vial was sealed with plastic film with small ventilation holes. For treatment one worm was kept per vial at different doses (10 earthworms/dose). Worms were kept on moist filter paper for three hours before being placed in test vials so they can void their gut contents. They were then washed and dried before use. During the test, vials were laid on their sides on trays. The test temperature was 20-25ºC. Tests were done in dark and for a period of 24 hours[20].

Range finding test

Broad dose range of 0.1mg, 1mg, 10mg, 100mg of sodium arsenite dissolved in 1 ml of distilled water was used for filter paper test.

Narrow range test

Sodium arsenite doses of 0.2, 0.4, 0.6 and 0.8mg/ml distilled water were used for filter paper test.

3. *Soil bioassay*

Standard vermicomposting medium of cow dung (50%) and soil (50%) was used to prepare an artificial soil bed for soil bioassay. The soil was characterized for physicochemical characteristics as pH, electrical conductivity, organic carbon, water holding capacity, phosphorus and chloride[21,22]. Sodium arsenite in doses 2, 4, 6, 8, 10mg//kg of mixture dissolved in distilled water was added to the mixture. About 1000gm of the mixture was taken in plastic containers of 1000ml capacity and 113.076cm^2 surface area. The moisture content was maintained at 35% of the dry weight and pH maintained at 7.0-7.5. In each test dose 10 adult earthworms were added in each container. The number of survivors in each test was recorded after 14 days.The control group was always set along with experimental group taking ten earthworms but no toxicant administration (only distilled water) in all the tests.

Results Analysis

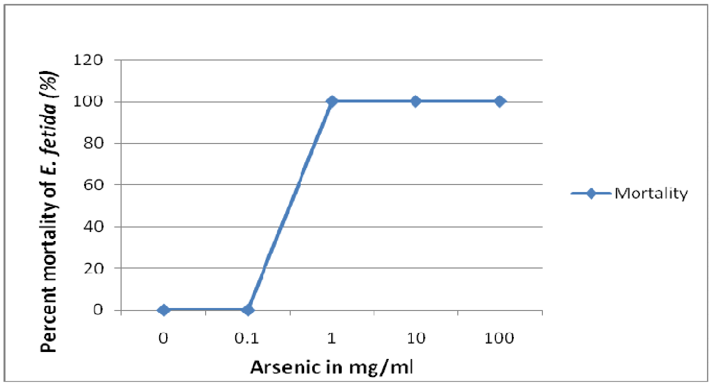
The results of spot application test and mortality were exhibited in percentage responses. The percent response and mortality at increasing doses were subjected to regression analysis to get a linear dose response curve. The EC_{50} and LD_{50} were computed from the dose response curve.

RESULTS

Range Finding Test

In the range finding test broad range of sodium arsenite was tested (0.1, 1, 10, 100 mg/ml) in filter paper test. There was no mortality in control and 0.1 mg/ml but at higher doses of 1, 10 and 100 mg/ml 100% mortality was observed after 24 hours (Fig 1). Thus for further study smaller dose intervals in the range of (0-1 mg/ml) was selected for spot rejection, contact filter paper and soil bioassay.

Figure 1
Graph showing percent mortality of E. fetida in filter paper test



Spot Rejection Test

In the spot rejection test there was increasing rejection of *Eisenia fetida* with increasing doses of sodium arsenite from 0.1 mg/ml to 0.7 mg/ml. At higher doses (0.7-0.9 mg/ml) the worm showed 100% rejection. In the untreated controls there was no rejection (Fig 2, 3). EC_{50} in spot rejection test was estimated to be 0.3 mg/ml of sodium arsenite concentration to *Eisenia fetida*.

Figure 2
Graph showing rejection of sodium arsenite by E. fetida in spot rejection test

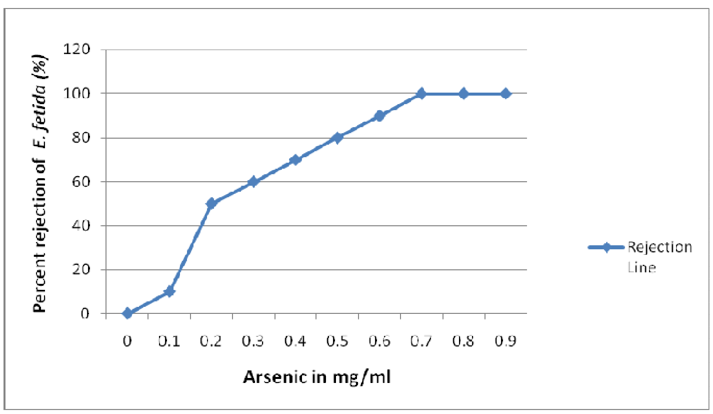
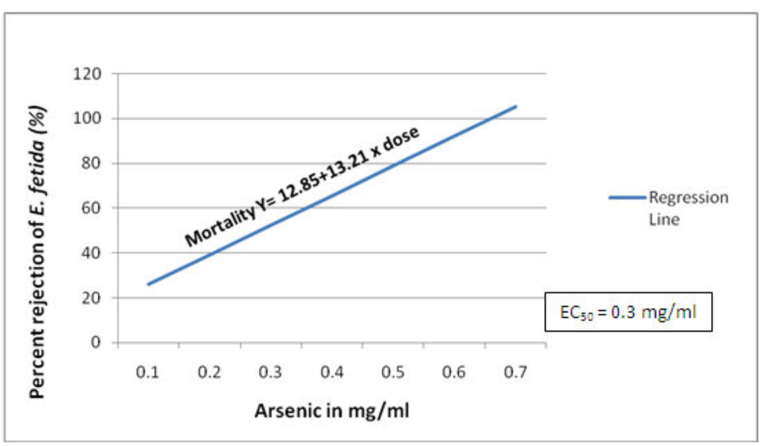


Figure 3
Regression line showing rejection of sodium arsenite doses after spot rejection test



Filter Paper Test
In the filter paper test there was increasing mortality of *Eisenia fetida* with increasing doses of sodium arsenite from 0.1 to 0.6 mg/cm^2. At higher doses of 0.7-0.8mg/ml, the worm showed 100% mortality. In the untreated controls there was no mortality after 24 hours of treatment (Table 3, 4; Fig. 3, 4, 5). LD_{50} was estimated as 0.4 mg/ml of sodium arsenite concentration to *Eisenia fetida*.

Figure 4
Graph showing percent mortality of E. fetida in filter paper test

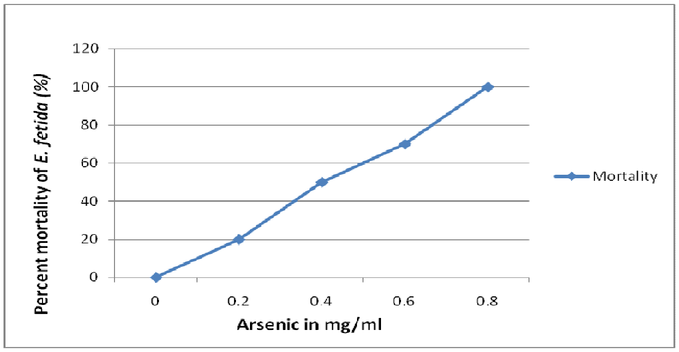
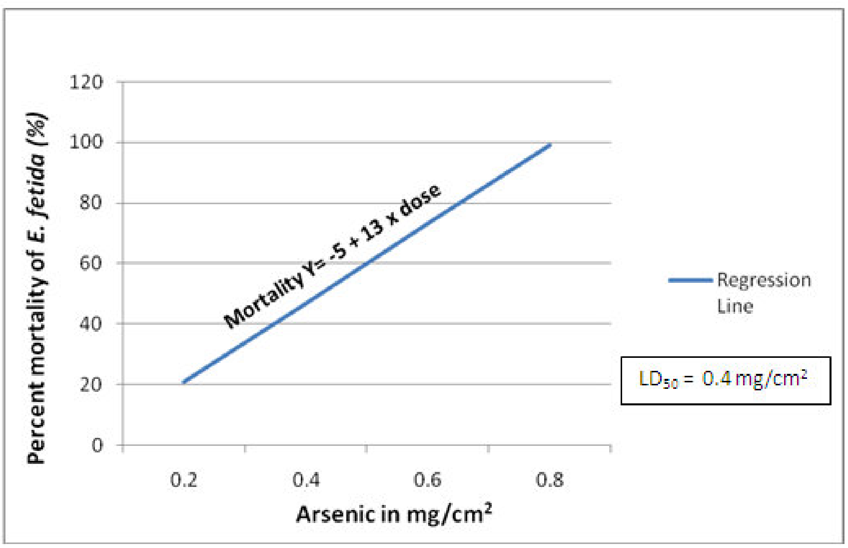


Figure 5
Regression line showing percent mortality of sodium arsenite doses after filter paper test



Soil Bioassay
The soil used for bioassay was considered to be normal on the basis of physico-chemical characterization (Table 1). In the soil bioassay there was an abrupt increase in the mortality of *Eisenia fetida* with increasing doses of sodium arsenite from 4mg to 6mg/ Kg of soil. At higher doses (6-10mg/Kg) the worm showed 100% mortality. In the untreated controls there was no mortality after 14 days of treatment (Fig 6, 7). LD_{50} was estimated as 5mg of sodium arsenite/Kg of soil to *Eisenia fetida*.

Table 1
Physico-chemical tests of soil sample & used for soil bioassay

SOIL SAMPLE	pH	ELECRICAL CONDUCTIVITY (mS)	ORGANIC CARBON (%)	ORGANIC MATTER (%)	WATER HOLDING CAPACITY (%)	CHLORIDE (mg/Lit)	AVAILABLE PHOSPHORUS (Kg/hact)
1.	7.05	0.484	0.721 ±0.2	1.243 ±0.2	70	53.175	286.46

Figure 6
Graph showing percent mortality of E. fetida in soil bioassay

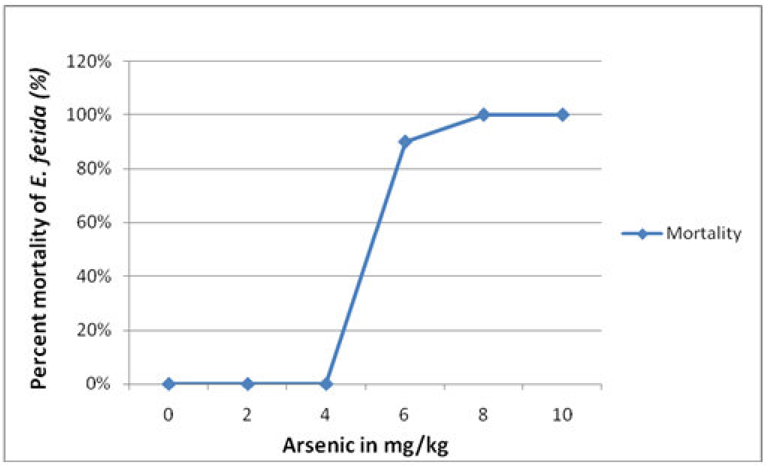
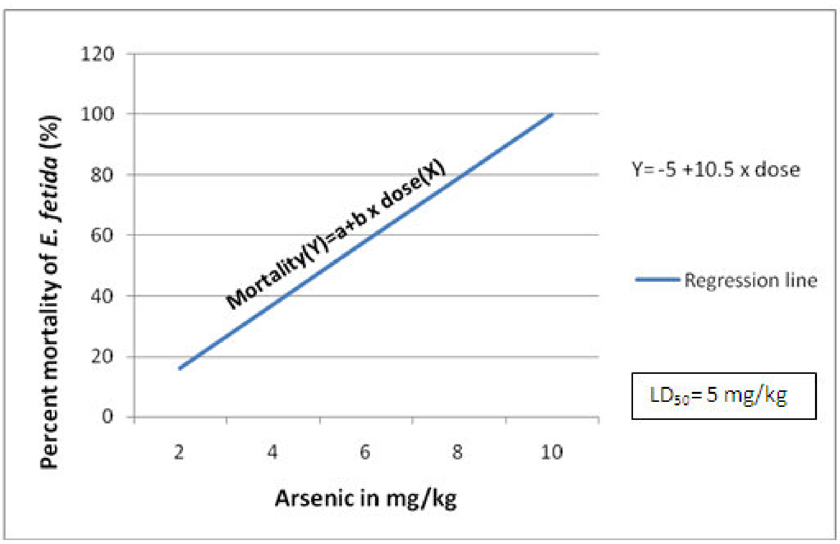


Figure 7
Regression line showing percent mortality of sodium arsenite doses after soil bioassay



DISCUSSION

The $EC_{50}$and LD_{50} in all the tests varied to some extent, this indicates that the toxicity apparently differs with different test methods. The spot rejection and contact filter paper test is initial screening technique of ecotoxicity since the toxicants are mainly absorbed through skin; but this does not represent the situation in soil[23,24]. More appropriate is the soil bioassay as it represents the earthworm natural environment as the toxicant is not only absorbed through skin but also through gut.The accumulation and toxicity of arsenic varies with field condition it has been observed that arsenic accumulation reduces in earthworm if the soil is supplemented with chloride and phosphate as arsenic forms complex with chloride and monovalent phosphate competes with arsenic for uptake through transporter mediated mechanisms in cells[25]. The effect of arsenic on growth and reproduction of *Lumbricus rubellus* has also been reported[26].The living organisms are always exposed to multitude environmental changes during their lifetime.These changes serve as external stimuli resulting in behavioural changes in organisms[27].The escape response of earthworm after exposure to any toxicants can clearly indicate its response to circumstances in which it lives. The neural mechanisms behind it can be mediated by a system of giant axons in the nerves which transmit the impulses at high speed[28].The mortality percent observed in contact filter paper and soil bioassay showed some difference at same dose. This can be attributed to slow acting toxicity of arsenic[29]. The mortality after exposure to sodium arsenite can be because of damage intestinal epithelium as observed in a study after exposure of trichlorobenzene to *Eisenia fetida* [6]. In a study conducted, when ecotoxicity of polycyclic musk was estimated at sublethal dose on *E. fetida*, the expression of superoxide dismutase, catalase and calreticulin genes was upregulated and annetocin and *hsp70* was downregulated[30]. Arsenic exposure in aquatic system has been reported to result in rupture of chromophores in fresh water fish *Channa punctatus*[31]. Toxicity of sodium arsenite has been reported to be cause by binding of arsenite to the sulphahydryl group of protein & there by disrupting its function[17,18].As an adaptive response to presence of arsenic in environment earthworms have been reported to accumulated arsenic in tissues in form of As V and As III as well as organic forms like Arsenobetaine and arsenosugars[32]. Various systemic and morphometric side effects of arsenic poisoning are associated with inhibition in nutrition absorption resulting in malnutrition and intestinal dysfunction in mice[33].

CONCLUSION

Based on the present experimental study on ecotoxicity of sodium arsenite on *Eisenia fetida*, it can be concluded that Sodium Arsenite is toxic to *Eisenia fetida* in dose of 0.1mg and above in contact assay and 1mg/Kg in soil bioassay. Therefore, it can be suggested that environmental soils which are containing 1mg/kg and higher doses of sodium arsenite will not be suitable for the growth of *Eisenia fetida* which is a common earthworm used for vermicomposting.

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