THE POPULATION BEHAVIOR OF EARTH WORM (*PHERITEMA POSTHUMA* KINBERG). UNDER THE INFLUENCE OF INDUSTRIAL WASTE

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Abstract

Earthworms are important component of our agricultural soil, food chain or ecosystem. Present study was conducted to explore the toxicity of industrial waste (if used as organic substitute on the field) on earthworm population. A four months green house experiment was conducted in Federal Urdu University where *Pheretima posthuma* (earth worms) was exposed to a series of industrial waste treatments. After the termination of experiment the number of adult, number of juvenile, number of cocoon and their body mass were calculated under the influence of two types of sludge and their ashes. The strongest impact of substrate contamination was exerted which significantly reduced the number of juvenile population at $p<0.001$ and body mass at $p<0.001$. The concentrations of all heavy metals in prepared polluted soil were greater than the permissible limits of WHO, while accumulation of Co and Pb in body mass of earth worms were determined above the WHO limits under both the ashes treatments. The Pearson’s correlation was evident significant relationship between the heavy metals contents in the substrate and their accumulation in earth worm’s tissues. It could be suggested that such types of industrial wastes should be banned contained high amount of heavy metals. Although *P.posthuma* is a good indicator of heavy metal, however this species may be used for vermicomposting of organic sludge with low concentration of heavy metals.

Introduction

Earth worms are the important member of soil fauna, aiding the maintenance of acration, water permeability, organic and mineral turn over in the soil. They are semi-terrestrial animals and are found in almost all soil types except for deserts and the areas permanently covered with ice (Gupta *et al.,* 2006). Earthworms play significant role in soil fertility by influencing decomposition processes like fragmentation of leaf litter and organic matter (Sehulman and Tuinov, 1999). According to Jhonson, (2002) earthworms play a vital role in the formation of fertile soil because soil microbial activity is also stimulated by the activities of earthworms (Binet *et al.,* 1998). Earthworms are the most considerable soil organisms and mostly used for the test of soil ecotoxicology because of their easy handling and measuring their growth parameters. In compression to other terrestrial invertebrates, earthworms are more susceptible to metal pollution and they are known to be a good indicator for metal contamination in soil (Lev *et al*., 2010).

The pollution of the heavy metal in soil consists of various sources like discharge of untreated industrial waste water and disposal of solid waste in open areas. The biggest sources of soil pollution are tannery, textile and electroplating industries. Earthworms have the capability to accumulate heavy metals from contaminated soil (Spurgeon and Hopkin, 1996 and Roseiszewska *et al.,* 2003). In terrestrial invertebrates mostly earthworms are used in the risk assessment for chemicals and their effects (Surgeon *et al.,* 2003). The contamination of heavy metals in soil may affect the reproductive capability of invertebrates, which is sensitive indicator of the animal’s conditions (Lapinski *et al.,* 2008). Depending on the stress resulting from the contamination the outcome may be described through survivability, reproduction or growth rate of individual (Lapinski *et al.,* 2008). Heavy metals are the most hazardous group of soil pollutants causes a serious problem because they can not be naturally degraded like organic pollutants and accumulate in different levels of food chain (Smejkalova *et al*., 2003).

In Pakistan, most of the industries discharge their untreated waste in running streams and solid waste like sludge containing heavy metals are also thrown in the vicinity of cites where these are agriculture fields nearby. Due to illegal discharge or dumping of the hazardous waste, heavy metals reach into the soil which may badly affected the population of earthworm. No work has been done on the bio-accumulation of heavy metal in earthworm species in Pakistan. Many birds depend on earth worms and these birds may be a part of human diet. However, accumulation of heavy metals in earthworms is consequently not only a threat to the earthworms themselves but also for the health of their preying organisms and human as well. So, the present study evaluated the effect of contaminated soil surface by spreading a layer of industrial sludge and its ash on the population of

Pheritema posthuma (earth worms). Their survivability and the accumulation of heavy metals in their tissues were investigated.

Materials and methods

A green house experiment was conducted in pots and various growth parameters and bio-accumulation of five heavy metals in *Pheretima posthuma* (earth worms) were observed. Four different types of industrial wastes namely chemical industry sludge, chemical industry ash, fiber industry sludge and fiber industry ash were collected from the dumping site of two industries. All the industrial wastes were chemically analyzed. Sandy loam was collected randomly with an auger from 0-20 cm layer from the barren area of Karachi. These samples were oven dried, crushed, sieved with 2mm sieve and pooled together to provide the homogenous medium for earth worm. Eight kg sandy loam with 50g dung manure was filled in fifteen pots sized 35cm in height and 30cm in diameter. Twenty adult earth worms were placed in each pot after weighing of each worm, approximately equal weight. A layer of industrial waste (Sludge and Ash separately, 200g per pot) were broadcast. Five treatments were made with three replicates including control. After four months quantitative analysis of adult worms, juvenile and cocoons were made, while fresh weight of worms and weight reduction percentages were also recorded. Five heavy metals including Zn, Fe, Co, Mn and Pb in soil (including all treatments) and tissues of earth worms were analyzed.

Preparation of soil and tissues samples

Soil and tissues samples were oven dried at 70°C until it reached constant weight. Dried soil samples were passed through a 10 mesh sieve, aggregate being broken gently by mortar and pestle. Samples were wet digested for heavy metals analysis following Berrow and Stein, (1983) and five heavy metals Zn, Fe, Co, Mn and Pb were analyzed by Atomic Absorption Spectrophotometer Model PG 990. Blank was also prepared following same method.

The data were subjected to soft wares COSTAT ver.3 and SPSS ver.10 for the analysis of variance Steel and Torrie, (1984); Duncan, (1955) multivariate analysis.

Results

The population of earthworm in Fiber industry sludge and Chemical industry sludge ash is described in Fig. 1. After four months number of adults, number of juvenile, number of cocoon, fresh weight and concentration of five heavy metals (Zn, Fe, Co, Mn and Pb) in soil and tissues of *Pheretima posthuma* were obtained and given in Table 1 to 3. The correlations between the number of earth worms and the concentration of the elements in the substrate were also examined. It was also estimated that fiber industry sludge contain high amount of organic matter (79%) while chemical industry sludge contained (68%) organic matter.

Table 1 shows the results of growth parameters of *Pheretima posthuma*. The result of ANOVA described the significance level of these parameters among treatments. Number of adult ($F=8.92$, $p < 0.01$), number of juvenile ($F=14.79$, $p < 0.01$), number of cocoons ($F=7.01$, $p < 0.01$) and fresh weight after four months ($F=53.76$, $p < 0.001$) were found significant. The order of the maximum toxic effect on these parameters was, fresh weight>number of juvenile>number of adult>number of cocoons. The numbers of adults ($p < 0.01$) and Juveniles ($p < 0.001$) were significantly reduced in all treatments except fiber industry sludge compared to control. Number of cocoons were significantly ($p < 0.01$) repressed in fiber industry ash and chemical industry ash treatments allied to control. The fresh weight was significantly reduced ($p < 0.001$) in all treatments as compared to control. Fiber industrial sludge showed better response on the mean values of number of adults (18 ± 2.02), number of Juvenile (19 ± 2.01), number of cocoon (10 ± 2.07) and fresh weight of four months worms (0.63 ± 0.04) compared to other treatments. Whereas, the lowest mean values of all parameters were recorded in chemical industry sludge-ash compared to other treatments.

Analysis of variance of heavy metals in soil

Table 2 presents the result of ANOVA for heavy metals contamination in soil after the experiment terminated. The results explained that all the heavy metals, Zn ($F=52.42$), Fe ($F=11.56$), Co ($F=51.71$), Mn ($F=315.0$) and Pb ($F=9.36$) at $p < 0.001$. However, Zn ($p< 0.001$), Co ($p < 0.001$) and Pb ($p < 0.001$) were significantly higher in all treatments compared to control. Significantly higher values of Fe ($p < 0.001$) was obtained in chemical industry ash treatment related to control, while Pb ($p < 0.001$) was significantly greater in fiber industry ash and chemical industry ash treatment compared to control. The maximum values of heavy metals in soil Zn (98.1 ± 4.40), Co (7.67 ± 0.64) and Mn (6.96 ± 0.27) were obtained in fiber industry ash treatment allied to other treatments. Similarly, Fe (98.16 ± 3.83) and Pb (3.28 ± 0.12) were recorded higher in chemical industry sludge-ash among treatments.

Heavy metal in soil

In general, all metals showed considerable variations among all treatments. The highest mean value of Zn was found in fiber industry ash while least was recorded in chemical industry sludge as compared to other treatments. The order of mean concentration of Zn among all treatments was observed as fiber industry ash > chemical industry ash > fiber industry sludge > chemical industry sludge. Iron was described in Figure 1b. The maximum mean concentration of Fe was found in chemical industry ash whereas the lowest was recorded in fiber industry sludge compared to other treatment. The sequence of higher concentration of Fe deposited in soil was described in Box whisker plots where chemical industry ash > chemical industry sludge > fiber industry ash > fiber industry sludge in that order. Figure 1c demonstrated the mean concentration of Co in box whisker plots. The greater mean values of Co were observed in fiber industry ash while chemical industry sludge showed lesser amount of Co in soil. Among treatments the order of the variation of Co in soil was defined as fiber industry ash > chemical industry ash > fiber industry sludge> chemical industry sludge. The higher concentration of Mn was found in fiber industry ash while the lowest was recorded in chemical industry sludge compared to other treatments of Mn in soil was in order fiber industry ash > fiber industry sludge > chemical industry ash > chemical industry sludge. Greater concentration of Pb was observed in chemical industry ash, while lowest in fiber industry sludge among treatments. The order of mean concentration of Pb among treatments was in order: chemical industry ash > fiber industry ash > chemical industry sludge > fiber industry sludge.

Heavy metals in tissues

Table 3 describes the result of ANOVA for accumulation of heavy metals in body of earth worms. All metals showed highly significant F-values of Zn (F=12.48), Fe (F=103.02), Co (F=59.59), Mn (F=73.18) and Pb (F=56.19) at $p < 0.001$. All the heavy metals Zn, Fe, Co, Mn and Pb ($p < 0.001$) were found significantly higher in all treatments compared to control. Zn 103.75 ± 5.35 µg/g, Co 63.51 ± 2.71 µg/g and Mn 109.3 ± 3.53 µg/g were obtained in fiber industry ash treatment, while Fe 100.62 ± 3.52 µg/g and Pb 51.09 ± 1.78 µg/g were significantly higher in chemical industry ash treatment compared to other treatments. The minimum accumulation of Zn 79.19 ± 2.70 µg/g, Co 38.05 ± 2.02 µg/g and Mn 85.1 ± 3.10 µg/g were found in chemical industry sludge treatment, while lowest values of Fe 41.43 ± 4.18 µg/g and Pb 31.31 ± 1.80 µg/g were in fiber industry sludge among other treatments.

All metals showed great variation in accumulation among treatments. The highest concentration of Zn was found in fiber industry ash whereas, lowest was in chemical industry sludge compared to other treatments. The order of the mean values of Zn among treatments was fiber industry ash > chemical industry ash > chemical industry sludge > fiber industry sludge. The order of mean concentration of Fe among treatments was chemical industry ash > chemical industry sludge > fiber industry ash > fiber industry sludge. The accumulation of Fe was found maximum in chemical industry ash compared to other treatments. The highest mean concentration of Co was obtained in fiber industry ash, while lowest in fiber industry sludge among treatments. All treatments were defined with the great accumulation as fiber industry ash > chemical industry ash > chemical industry sludge > fiber industry sludge in that order. Similarly the maximum amount of Mn was also deposited in earth worms dry biomass through chemical industry ash treatment, whereas the minimum amount was found in chemical industry sludge of Mn that fiber industry ash > chemical industry ash > chemical industry sludge > fiber industry sludge Pb accumulation in earth worm tissues showed slightly different order: chemical industry ash > fiber industry ash > chemical industry sludge > fiber industry sludge. The highest accumulation of Pb was obtained in fiber industry ash treatment.

Correlations

The analysis showed that for each of the studied elements, the correlations between the concentration of the elements in the substrates and their accumulation in the body of earth worms were statistically significant. The Pearson’s coefficient for zinc accumulation was maximum $r = 0.977$, while minimum for Mn was $r=0.785$ (Fig. 2, a-e).

Table 1 Duncan's multiple range test of growth parameters of *Pheritema posthuma.*

Treatments	Number of adult	Number of juvenile	Number of cocoon	fresh weight of 4 months worms (g)
F – values	8.92**	14.79***	7.01**	53.76***
Control	21±2.02 a	22±2.60 a	12±1.15 a	0.78±0.03 a
Fiber industry sludge	18±2.02 ab	19±2.01 ab	10±2.07 a	0.63±0.04 b
Fiber industry ash	8±1.73 c	5±0.88 c	5±1.45 bc	0.37±0.01 d
Chemical industry sludge	13±2.30 bc	15±2.60 b	8±1.73 ab	0.52±0.02 c
Chemical industry ash	6±2.60 c	5±1.08 c	2±0.33 c	0.30±0.01 d
Lsd	6.80	6.17	4.60	0.08

Note: *=p<0.05, **=p<0.01, ***=p<0.001, ns=non-significant. Figures followed by same letter in a column are non-significantly different as given by the Duncan's multiple test, Lsd = least significant difference.

Table 2. Heavy metal contamination in soil (Mean ±SE)

Treatments	Zn (mg/kg)	Fe (mg/kg)	Co (mg/kg)	Mn (mg/kg)	Pb (mg/kg)
F – values	52.42***	11.56***	51.71***	315***	9.36***
Control	21.03±2.94 c	67.06±3.02 b	0.98±0.03 c	1.34±0.03 e	1.08±0.33 c
Fiber industry sludge	58.06±5.36 b	69.13±2.33 b	4.11±0.34 b	5.37±0.07 b	1.10±0.37 c
Fiber industry ash	98.1±4.40 a	72.03±4.73 b	7.67±0.64 a	6.96±0.27 a	2.80±0.50 ab
Chemical industry sludge	53.13±1.90 b	74.1±4.15 b	3.33±0.12 b	2.10±0.05 d	1.90±0.03 bc
Chemical industry ash	88.13±5.35 a	98.16±3.83 a	4.35±0.06 b	3.77±0.02 c	3.28±0.12 a
Lsd	13.31	11.70	1.05	0.40	1.02

Note: ***=p<0.001, ns=non-significant. Figures followed by same letter in a column are non-significantly different as given by the Duncan's multiple test, Lsd = least significant difference.

Table 3. Heavy metal accumulation in *P.posthuma* tissues.

Treatments	Zn (µg/g)	Fe (µg/g)	Co (µg/g)	Mn (µg/g)	Pb (µg/g)
F – values	12.48***	103.02***	59.59***	73.18***	56.19***
Control	63.03±4.88 c	29.58±1.69 e	22.20±1.72 d	37.30±0.81 d	21.23±1.33 d
Fiber industry sludge	79.19±42.70 b	41.43±4.18 d	38.05±2.02 c	85.10±3.10 c	31.31±1.80 c
Fiber industry ash	103.75±5.35 a	55.92±2.43 c	63.51±2.71 a	109.3±3.53 a	46.41±1.77 a
Chemical industry sludge	90.17±4.14 ab	79.87±1.28 b	42.40±1.62 c	88.06±4.51 bc	41.06±1.21 b
Chemical industry ash	98.83±5.45 a	100.62±3.52 a	53.80±1.91 b	97.27±2.87 b	51.09±1.78 a
Lsd	14.56	8.94	6.42	10.10	5.04

Note: ***=p<0.001, ns=non-significant. Figures followed by same letter in a column are non-significantly different as given by the Duncan's multiple test, Lsd = least significant difference.

Discussion

Ash treatments of fiber industry and chemical industry were highly toxic to the growth of earthworms compared to sludge treatments of the same industries. The growth parameters of earthworms were significantly suppressed by ash treatments. This might be due to high ash toxicity because ashes contained no organic matter but a high concentration of heavy metals compared to sludge treatments. Ashes provide a toxic substrate for earthworms resulted in rapid mortality for test species. *P. posthuma* is an anecic species which dwelled on near to surface of the substrate and actively ingested large amount of soil treated with ashes, automatically exposed to heavy metals through their intestine as well as through skin therefore, heavy metal accumulated from the soil in their body and bio-concentrated (Cortet *et al*., 1999; Morgan and Morgan, 1999). They may serve as bio-indicators of soil contaminated with pesticides and heavy metals (Spurgeon and Hopkin, 1996). Honda and Tatsukawa, (1984) reported that the accumulation of Fe, Mn, Zn, and Co in earthworm depended primarily on the metabolic turnover of *P. posthuma* whereas; the accumulation of Pb is dependent upon the age or exposure time. According to Van Straalen *et al.*, (1989) the contamination of heavy metals over a long time, may alter the ultimate survival of population of earthworms. Cheng and Wong, (2002) also found increase of heavy metal availability in *Pheretima* species with time. Many workers Rhee, (1977); Malecki *et al.*, (1982); Bengtsson *et al.*, (1986) reported that the heavy metals effect on the survival and cocoon production which increases the rate of mortality.

Cocoon production was also significantly inhibited in both types of ash treatments. Ismail, (1997) reported that the cocoon production in favorable conditions takes six weeks to six months and one pair of earth worm can produced 100 cocoons. The results of the present study did not trail the findings of Ismail, (1997) it might be due to high toxicity of bio-accumulated metals specially Pb and Zn. The similar toxic effect of these heavy metals on cocoon production was also studied by Haghparast, (2009). According to Kale, (1995) organic matter which provided a nutrient rich medium to support higher population and produced more cocoons. Lev *et al*., (2010) reported that the higher level of Zn decreased the growth of earthworm. In our study the heavy metals in treatments also inhibited the fresh body mass of *P. posthuma*. Similar results were presented by Malecki *et al*., (1982) and Neuhauser *et al*., (1984). They indicated that the body mass of earth worms decreased with the increased in contamination of the substrate. The decrease in body mass might be associated with a lower availability of food and due to the heavy metals contaminated substrate. The study revealed statistically significant differences between masses of earth worms exposed to various concentrations of heavy metals. Gish and Christensen, (1973); Van Hook, (1974) have shown that earth worms accumulated Pb from soil but can survive in conditions of considerable metal pollution. Earthworm showed higher respiration and excretion (Pradhan and Mishra, 1986).

The number of adults, juvenile, cocoons in fiber industry sludge showed non-significant relationship as compared to controls might be due to the presence of organic matter. According to Haghparast *et al.*, (2009) the addition of organic matter to soils reduced the toxic effects of heavy metals to earthworms. Edward and Lofty, (1982) and Lacerda and Abrao, (1984) also stated that earthworm abundance and biomass was greatest in plots having manure. Haghiri (1974) explained the chelating property of organic matter which combined certain groups of heavy metals and fixes them. The fixation of heavy metals resulted least bio-accumulation in earth worms tissues whereas number of adults and juveniles were significantly reduced in chemical industry sludge. However, chemical industry sludge also contained organic mass but it might be possible that the presence of high amount of heavy metals retrograde these two growth parameters.

The results concluded that the degree of accumulation depends on the nature of the waste and the species concerned. It could be suggested that this species of earthworm behaved as bio-indicator though it was time consuming indices of environmental pollution, as also suggested by Morgan and Morgan, (1999). According to Lev *et al*., (2010) earthwworms showed increasing bioaccumulation of Zn with increasing Zn soil concentrations. The concentration of Co and Pb was found higher than the permissible limits of WHO (1987) in body mass of earth worm. In present study, Pb bioaccumulated in earthworms. The predation of earthworms could constitute a major pathway for the entry of heavy metals specially Pb into the food chain.

It was concluded that in substrate with a higher concentration of heavy metals the juvenile population is more affected by the heavy metal pollution. The survived earthworms contained high amount of heavy metals. Therefore, sludge/ash should not be used as organic substrates. The dumping of wastes in open areas will disturb the ecosystem balance and would be the health hazardous to human too.

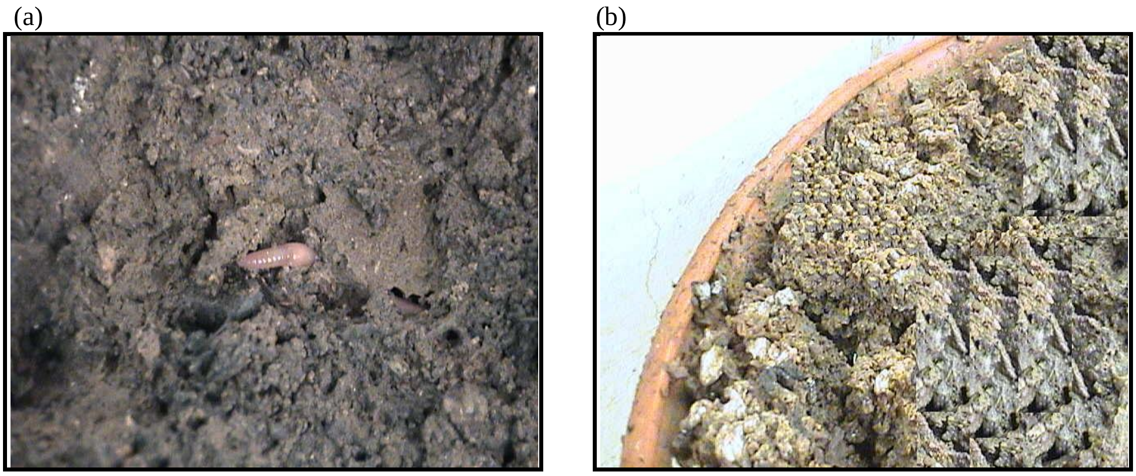

Figure 1. Spread sheet of a) fiber industry sludge and b) chemical industry sludge ash containing earthworms.

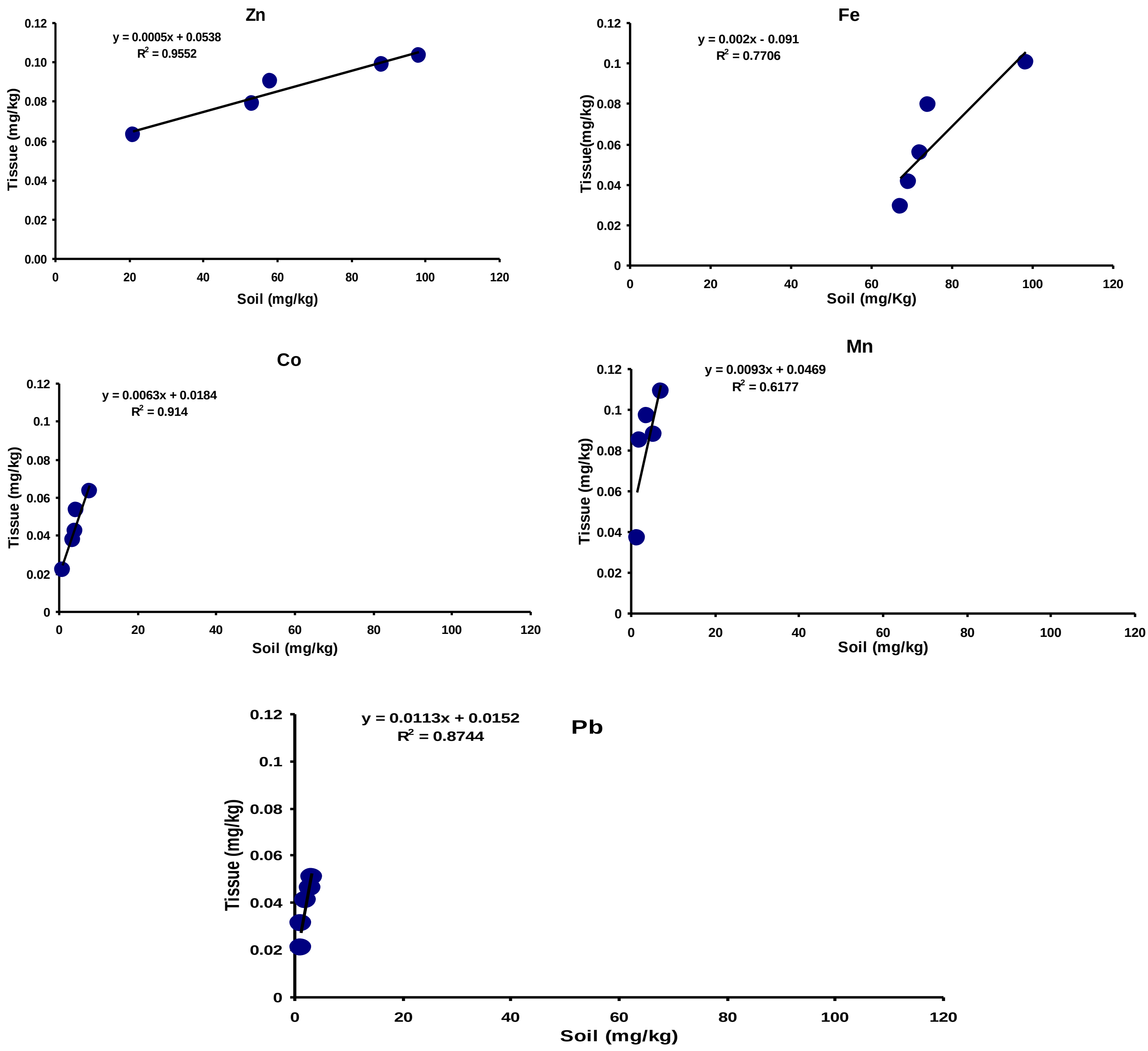

Figure 2 (a-e). Correlation of heavy metals contents of soil and the tissues of *P . posthuma.*

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