ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Bioaccumulation of heavy metals by earthworms from contaminated soils of vegetable fields in the vicinity of Buddha Nullah, Ludhiana, Punjab

Sureakha Koul[1], Devinder Kaur Kocher[1]*and Dhanwinder Singh[2]

1. Department of Zoology, Punjab Agricultural University, Ludhiana-141004Punjab, India.
2. Department of Soil Science, Punjab Agricultural University, Ludhiana-141004Punjab, India.

Manuscript Info

Manuscript History:

Received: 14 November 2015
Final Accepted: 15 December 2015
Published Online: January 2016

Key words:
Bioaccumulation; Bio-indicator; Buddha Nullah; Earthworms; Heavy metal contamination.

Corresponding Author

Devinder Kaur Kocher.

Abstract

The present study was aimed to analyse the status of heavy metals viz, Lead, Manganese, Cadmium, Nickel, Zinc, Chromium and Arsenic in soils and tissues of earthworms at vegetable crop fields in the vicinity of Buddha Nullah (a seasonal water stream highly contaminated with domestic and industrial wastes), Ludhiana district, Punjab. Three species of earthworms viz; *Metaphire posthuma* (Vaillant), *Metaphire peguana* (Rosa) and *Lampito mauritii* (Kinberg), inhabiting in these areas were found to accumulate certain amount of metals in their tissues. Three replicates each of soil and earthworm samples were collected from four study sites in the vicinity of Buddha Nullah (as contaminated sites) and one site at PAU (as reference site). The heavy metal concentrations in soil samples and tissues of earthworms collected from vegetable fields lying near Buddha Nullah site were significantly higher than that obtained from the reference site. Bioaccumulation factor for heavy metals was found to be higher in the *L.mauritii* as compared to other two species of earthworms, indicating it could serve as a possible bio-indicator of heavy metal contamination.

Introduction:-

Earthworms form an important invertebrate community component in most soils in terms of their contribution to overall below ground biomass and play a predominant role in the formation and maintenance of soil structure, soil fertility and organic matter dynamics through nutrient cycling and decomposition of residues (Dominguez et al., 2009). The presence as well as the absence of earthworm species and their relative abundance provide a gross indication of the health of the soil (Rochfort et al., 2009). Earthworms accumulate a considerable content of metals in their tissues and could serve as useful biological indicators of contamination in soil (Suthar et al., 2008). Even the accumulation of metals may depends largely on the interaction of the earthworm with local edaphic factors, such as pH, organic matter content, etc. (Lukkari et al., 2006). Heavy metal pollution of terrestrial ecosystems has long been recognized as a serious environmental concern. Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities, and humans are exposed to them through various pathways (Wilson and Pyatt, 2007). Heavy metals are considered serious pollutants because they are toxic and non-degradable, thus accumulation of heavy metals in soil poses many risk to humans and the ecosystem (Odoh et al., 2011). The total heavy element content in soil is frequently used as a criterion for defining soil contamination. Soluble metal concentrations are the best descriptors of bioaccumulation in earthworms (Peijnenberg et al., 1999). The implication associated with heavy metal contamination is of great concern particularly in agricultural production system (Uzoma et al., 2013). One of the most peculiar and special behaviour of earthworms is to accumulate heavy metals in their tissues and are enough sensitive to contaminants, which make them good bioindicators (Nahmani and Lavelle, 2002). They are important organisms in terms of soil functionality and consequently play a key role in terrestrial eco- toxicological risk assessment (Weeks et al., 2004).

Buddha Nullah is considered a seasonal water stream and highly contaminated with domestic and industrial sewage and runs through the Sutlej river of Punjab, after passing through highly populated and industrialized district Ludhiana. Hundreds of tonnes of raw sewage from Ludhiana flow into the Sutlej every day and are distributed all over the state through irrigation canals (Anonymous, 2008). About 80% of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems, providing an early warning of deterioration of soil quality (Bustos and Goichochea, 2002). Hence, the present research was aimed to study the status of heavy metals in soils and bioaccumulation in tissues of earthworms at selected vegetable fields inhabiting the vicinity of Buddha Nullah, and compare these parameters with reference site at Punjab Agricultural University, Ludhiana.

Materials and methods:-

Four vegetable crop fields situated at an average distance of 2-3 kms from Buddha Nullah selected to conduct the present study were; site I (Dairian village), site II (Jassian village), site III (Choorpur village) and site IV (Balloke village). Another site V was selected at Punjab Agricultural University vegetable farms as reference site. At all the sites soil samples were randomly collected from top 0-15 cm (in triplicate) and then hand sorted to remove root and litters. The samples were air dried and stored in labelled polythene bags prior to analysis.

2.1 Physico- chemical properties of soils

The soil samples from each sampling site were analysed for soil pH using Potentiometric method (Jackson, 1973), Electrical conductivity by the method of Richard (1954), soil organic matter contents by the method of Walkley and Black (1934) and Calcium carbonate was determined by the method of Puri (1930), Biota-to-soil accumulation factor in the earthworms was calculated by the formula given by Cortet et al. (1999).

2.2 Heavy metal estimation in soils

The soil samples were analysed for total heavy metal analysis of Pb, Cd, Ni, Cr, Zn, As, and Mn by digesting 1gm sample of soil sample with 12 ml of Aqua Regia (3:1 $HCl:HNO_3$) in Teflon microwave digestion vessel. Digestion was conducted at 100% of power (1600 watts) for 10 minutes till the temperature of the mixture reached to 160ºC.

2.3 Earthworm collection and preparation for heavy metal estimation

The earthworms collected from the study areas were thoroughly washed with distilled water and were kept in petri dishes (one animal per dish) having Whatman No. 1 filter paper and a few drops of water. They were kept at 14ºC for 7 days and the filter paper was changed to allow complete evacuation of the gut contents. The earthworms were killed by freezing and then they were oven dried by heating at 70ºC for 48 hours. After cooling the samples were placed in a desiccator, their dry weight was measured with the help of a balance. The dry earthworms were crushed and then 100 mg of sample was mixed with 4 ml of concentrated HNO_3 + 2 ml of concentrated HCl for 12 hours, and was heated progressively to 150ºC in a closed Teflon bottle in a micro-wave oven for 2 hours. After cooling at ambient temperature, the solution was made up to 50 ml with ultrapure distilled water.

2.4 Analysis of heavy metals in soil samples and earthworm tissues

Already prepared digested soil samples and tissue samples were analyzed for total Pb, Cd, Ni, Cr, Zn, Fe, Cu and Mn after appropriate dilutions using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICAP-AES) in the laboratory. Concentration of heavy metals in these samples was calculated by the following formula.

$$\text{Concentration of heavy metals (mg/kg)} = \frac{\text{Reading from ICAP-AES} \times \text{Volume of digest}}{\text{Weight of sample}}$$

2.5 Statistical analysis

The concentration of heavy metals in contaminated soil samples at Buddha Nullah and non-contaminated soil samples at PAU and in tissues of different earthworm species collected from respective sites were compared by using t-test. All data was calculated by using SPSS version 16.0 program for windows.

Results:-

The soil samples of the vegetable fields (sites I, II, III and IV) in vicinity of Buddha Nullah was found to have slight alkaline nature, as the pH value ranged from 7.90±0.06 to 8.30±0.06 at these sites. Average soil pH observed at Buddha Nullah sites was 8.10±0.11, which was statistically non-significant with that of reference site at PAU fields (8.37±0.15). Electrical conductivity (dSm^{-1}) in soil samples collected from the vegetable fields near Buddha Nullah was 0.30±0.06, which was also statistically non-significant with that of PAU (0.11±0.01) reference field. The percent organic matter at PAU reference field was 1.41±0.04, which was statistically higher as compared to that Buddha Nullah sites (1.17±0.10). Content of $CaCO_3$ (%) was found to be significantly low i.e 1.13±0.24 at PAU site in comparison to Buddha Nullah sites (3.97±0.45) as shown in Table 1.

The concentration of Arsenic, Cadmium, Chromium, Manganese, Nickel, Lead, and Zinc were found to be statistically higher (at 5% level of significance) in the soil samples collected from vegetable field areas (sites I, II, III and IV) in the vicinity of Buddha Nullah as compared to their respective concentration at PAU reference site. Maximum concentration of Arsenic (mg/kg) i.e 7.05±0.29 was found in soil samples taken from vegetable fields of Choorpur followed by Balloke (6.34±0.67), Jassian (5.95±0.32) and Dairian (5.14±0.59) villages near Buddha Nullah. On an average, at Buddha Nullah sites, Arsenic concentration (mg/kg) was found to be statistically higher i.e 6.12±0.40 as compared to that of reference site at PAU having 3.25±0.13. Concentration of Cd was found to range from 0.68±0.01 to 0.82±0.01 mg/kg with its average value 0.77±0.03 mg/kg in soil samples of Buddha Nullah sites and this concentration was statistically higher in comparison to that of reference fields at PAU (0.50±0.07 mg/kg). Average concentration of Cr (mg/kg) in soils at Buddha Nullah sites was 51.31±2.55, which was significantly higher as compared to its content found in soils at reference site PAU (31.24±2.81 mg/kg). Statistically higher value of Mn (292.33±6.47 mg/kg) was found in soil samples collected from vegetable fields lying near the Buddha Nullah as compared to that of PAU reference site, where Mn was found to be 168.21±11.15 mg/kg. Similarly statistically higher concentration of other tested heavy metals i.e Ni, Pb and Zn were observed at Buddha Nullah sites in comparison to that of PAU reference vegetable fields (Table 2). When the concentration of the heavy metals detected in the soil samples collected from the vegetable fields near Buddha Nullah were compared with the SEPA limits of these metals in soils, their levels were found to lie below the permissible limits (SEPA, 1995), except the concentration of Cd (0.77±0.03 mg/kg) which was higher than its permissible limits (0.6 mg/kg).

Three species of earthworms viz., *Metaphire posthuma*, *Metaphire peguana* and *Lampito mauritii* have been reported from the vegetable fields lying in the vicinity of Buddha Nullah as well as PAU fields (Koul et al., 2015). Analysis of various heavy metals like As, Cd, Cr, Mn, Ni, Pb and Zn in tissues of *M. posthuma* collected from sites I, II, III, and IV lying near the Buddha Nullah showed higher concentration of all these metals in comparison to the tissues of *M. posthuma* collected from the reference site fields at PAU. Out of the seven heavy metals, the concentration of four heavy metals i.e As, Mn, Ni, and Pb was found higher (at1% and 5% level of significance) in the tissues of *M. posthuma* collected from Buddha Nullah sites as compared to that of PAU reference site. Average concentration of As (mg/kg) was 12.80±2.04 and 0.30±0.01, Mn was 240.02±45.68 and 19.60±0.02, Ni was 21.11±5.48 and 1.75±0.01 and Pb was 20.69±4.45 and 1.93±0.01 in the tissues of *M. posthuma* collected from the Buddha Nullah area sites and PAU reference site respectively (Table 3). The analysis of various heavy metals in tissues of *M. peguana* collected from sites I, II, III, and IV lying near the Buddha Nullah showed higher concentration of these metals in comparison to the tissues of *M. peguana* collected from the reference site fields at PAU. Out of the seven heavy metals, the concentration of four heavy metals i.e As, Mn, Ni, and Pb was found to be higher (at 1% and 5% level of significance) in the tissues of *M. peguana* collected from Buddha Nullah sites as compared to that of PAU reference site. Average concentration (mg/kg) of As was 11.45±3.63 and 0.57±0.01, Mn was 179.20±94.32 and 26.09±0.01, Ni was 13.86±6.34 and 2.10±0.01 and Pb was 20.79±9.39 and 2.36±0.01 in the tissues of *M. peguana* collected from the Buddha Nullah area sites and PAU reference site respectively (Table 4). Concentrations of various heavy metals in tissues of *L. mauritii* collected from sites I, II, III, and IV lying near the Buddha Nullah were also found to be higher in comparison to that of reference site fields at PAU. Out of the seven heavy metals, the concentration of four heavy metals i.e As, Cr, Pb and Zn was found to be higher (at 1% and 5% level of significance) in the tissues of *L.mauritii* collected from Buddha Nullah sites as compared to that of PAU reference site. Average concentration of As (mg/kg) was 11.24±5.57 and 0.14±0.00, Cr was 38.43±22.48 and 0.57±0.00, Pb was 31.66±16.37 and 1.08±0.00 and Zn was 170.06±85.17 and 3.84±0.00 in the tissues of *L.mauritii* collected from the Buddha Nullah area sites and PAU reference site respectively (Table 5).

Bioconcentration factor (BCF) for heavy metal accumulation in tissues of various heavy metals viz; As, Cd, Cr, Mn, Ni, Pb and Zn in tissues of *M. posthuma* collected from sites I, II, III, and IV lying near the Buddha Nullah showed higher value of BCF for these metals in comparison to the tissues of *M. posthuma* collected from the reference site fields at PAU. Four heavy metals i.e As, Mn, Ni, and Pb were found to have higher BCF value (at 1% and 5% level of significance) in the tissues of *M. posthuma* collected from Buddha Nullah sites as compared to that of PAU reference site. Average value of BCF for As was 1.88±0.25 and 0.09±0.00, Mn was 0.95±0.26 and 0.12±0.00, Ni was 1.02±0.27 and 0.15±0.00 and Pb was 1.05±0.22 and 0.19±0.00 were accumulated in the tissues of *M. posthuma* collected from the Buddha Nullah area sites and PAU reference site, respectively (Table 6). Bioconcentration factor of various heavy metals viz; As, Cd, Cr, Mn, Ni, Pb and Zn in tissues of *M. peguana* collected from sites I, II, III, and IV lying near the Buddha Nullah showed its higher value for these metals in comparison to the tissues of *M. peguana* collected from the reference site fields at PAU. Four heavy metals i.e As, Mn, Ni, and Pb were found to have higher BCF value (at 1% and 5% level of significance) in the tissues of *M.peguana* collected from Buddha Nullah sites as compared to that of PAU reference site. Average value of BCF for As was 1.85 ±0.63 and 0.18±0.00, Mn was 0.60±0.35 and 0.16±0.00, Ni was 0.71±0.35 and 0.21±0.00 and Pb was 1.07±0.35 and 0.23±0.00 in the tissues of *M. peguana* collected from the Buddha Nullah area sites and PAU reference site, respectively (Table 7). Bioaccumulation factor of various heavy metals viz; As, Cd, Cr, Mn, Ni, Pb and Zn in tissues of *L. mauritii* collected from sites I, II, III, and IV lying near the Buddha Nullah showed higher value of BCF for all these metals in comparison to the tissues of of *L. mauritii* collected from the reference site fields at PAU. Four heavy metals i.e As, Cr, Pb and Zn were also found to have higher BCF value (at 1% and 5% level of significance) in the tissues of *L. mauritii* collected from Buddha Nullah sites as compared to that of PAU reference site. Average value of BCF for As was 2.26±1.31 and 0.04±0.00, Cr was 0.72±0.46 and 0.02±0.00, Pb was 1.68±1.04 and 0.10±0.00 and Zn was 2.25±1.28 and 0.10±0.00 in the tissues of *L.mauritii* collected from the Buddha Nullah area sites and PAU reference site, respectively (Table 8). BCF for accumulation of various heavy metals was found to be higher in *L. mauritii* species of earthworm as compared to *M. posthuma* and *M. peguana* (Fig 1).

Discussion:-

The soil pH influences the cation mobility and regulates the solubility of toxic trace metals in soil. The mobility and availability of trace metals decreases as the pH approaches neutral or rises above 7 in the soils and most of the toxic trace metals tend to be available in acidic pH (Rodriguez et al., 2008). Soil EC (dS m^{-1}) <0.8 is said to be normal, 0.8-1.6 is critical for salt –sensitive plants, 1.6-2.5 is critical for salt- tolerant plants and >2.5 is injurious to all plants according to standard values of soil components (Awashthi, 2000). Soil organic matter is the most important indicator of soil quality and in addition to acting as a store house of the plant nutrients, it plays a major role in nutrient cycling. The soil organic matter below 0.40 is said to be low, 0.40-0.75% is medium, and above 0.75% is high according to standard values of soil components (Awashthi, 2000). Nazif et al. (2006) studied that alkaline soils were found to have rich amount of organic matter. Rattan et al. (2005) observed a variation of OM content from 0.14-0.76 % (average 0.39%) in the study areas. Singh and Kumar (2006) observed that the soils loaded with toxic trace metals in Delhi were found to have 0.5-1% of OM value. An excess of calcium carbonate reduced the availability of some trace metals in soils by introducing too much calcium on to the soil complex (Sanchez-Martin et al., 2007). The soils of the study area were moderately calcareous in nature. Though all the selected vegetable fields lying in the vicinity of Buddha Nullah (sites I, II, III and IV) were found to be highly contaminated with different heavy metals in comparison to PAU reference site, yet the level of contamination of all these tested heavy metals was within the permissible limits (SEPA, 1995; Awashti, 2000) as compared to that of PAU reference site. The average concentration (mg/kg) of all the determined heavy metals i.e. Arsenic (6.12±0.40), Cadmium (0.77±0.03), Chromium (51.31±2.55), Manganese (292.33±6.47), Nickel (19.27±0.72), Lead (19.28±0.75) and Zinc (73.41±3.13) estimated in soil samples at Buddha Nullah sites and PAU site was found to be within the permissible limits except cadmium which was observed to be statistically higher in the soil collected from vegetable field areas (sites I, II, III and IV) lying in the vicinity of Buddha Nullah as compared to that of PAU reference site. Ayeni et al. (2010) have also observed similar results where concentrations of toxic trace metals in soil were found below permissible limits. Elevated level of metals in soils at different sites near Buddha Nullah is in agreement with contamination of soil with heavy metals in different sites of Avonmouth, southwest England (Spurgeon and Hopkin, 1996). The concentration of heavy metals in soil samples near Buddha Nullah has also been reported by Kaur and Sangha (2014) and Kaur (2015). The concentrations of heavy metals lower than the safer limits may be due to the continuous removal of these metals by the vegetables grown in these areas, also continuous cultivation and regular absorption by vegetables possibly keep the concentration of trace metals in soil within safe limits. The results indicated that metal concentration of Cadmium was above the Environmental Quality Standards set by the State Environmental Protection Administration (SEPA, 1995) at all the study sites and were below at the reference site (Table 2). However, none of the soils in this study exceeded the maximum permissible concentration levels for Cu and Zn as also reported by various researchers, (Kabata-Pendias and Pendias, 1984; SEPA, 1995; Awashthi, 2000).

Soft bodied soil dwelling organisms are exposed to metals either through direct dermal contact with metals in soil solution or by ingestion of bulk soil or specific soil fraction (Lanno et al., 2004). Almost every type of soil contains individuals of at least one type of earthworm Lumbricidae species. They are numerous large bodied individuals, which are sensitive enough to contaminants, thus making them good bio- indicators (Cortet et al., 1999). Because of limited mobility they have adapted to life in a certain soil depth under certain soil condition. The results showed that the concentration of the heavy metals in the earthworm tissues were higher than soil samples from the same sites where as earthworm tissues and soil samples from the reference site showed less concentration and similar findings were reported by Olayinka et al. (2011), who worked on earthworm *Hyperiodrilus africanus* in uncontaminated soils. The concentration of Mn was highest for all the metals analysed in the soil samples. This trend of highest Mn concentration obtained in this study is similar to the reported work of Anderson and Laursen (1982), who worked on *Lumbricus terrestris* in uncontaminated soil and also Olayinka et al. (2011) who worked on earthworm *H. africanus* in uncontaminated soils. Results showed that the metals found in soils are in very low concentration and are slowly bioaccumulated by the worms within their tissues. By definition the higher the BCF value, the more chemicals are taken up and the higher the potential risk regarding adverse effects on the organism itself and at higher tropical levels. The bioconcentration factor (the ratio of metal concentration of earthworm tissue and the soil) is cited as indicator of bioavailability in soil. So the amount of accumulation is relatively low because the accumulation depends on the concentration of metals in soils. The results showed that among the earthworms, *L. mauriti* was found to be better at sequestering metals within its tissues. In the present work three earthworm species viz., *M. posthuma*, *L. mauritii* and *M. Peguana* were used for accumulation of heavy metals in their tissues at the study sites. All the three species showed higher metal concentration in the selected vegetable fields lying the vicinity of Buddha Nullah (contaminated site) as compared to the PAU (reference site). This may be due to chemical change which occur in the alimentary tract of earthworms and hence render various metals more available to plants. It may also be as a result of in built earthworm body mechanism which reduces the rate at which the earthworms absorb heavy metals from contaminated soils. Mineralization of dead earthworms also releases accumulated heavy metals back to the environment (Morgan and Morgan, 1993). The worms accumulate metals within their tissues by binding with some metalloprotiens called as metallothioneines. Generally earthworms need to consume great amount of soil to achieve their daily nutrition during which the digestive process liberates heavy metals in their free forms in the gut lumen (Suthar, 2007). These freely available metals are then absorbed by the gut epithelial lining. So, in this way earthworm tends to accumulate considerable amount of the heavy metals within the cells in alimentary canal (Suthar et al., 2008). The accumulation behaviour of heavy metals in earthworms and their sensitivity to these pollutants can vary from species to species (Hobbelen et al., 2006) as also seen during the present study, like *L. mauritii* earthworm species showed higher accumulation of heavy metals in its tissues as compared to *M. posthuma* and *M. peguana*, so may act as a better indicator of heavy metal contamination of soil. Therefore, earthworms may be used as indicators of environmental heavy metal pollution. Although the amount of metals accumulated within earthworm tissues is partly dependent on the absolute concentration of the metals within the given soil, yet it is strongly co-determined by physiochemical edaphic interactions, including factors such as pH, calcium concentration and organic matter (Morgan and Morgan, 1993).

This study showed the accumulation of some amount of heavy metals in earthworms inhabiting the vegetable crop fields in the vicinity of Buddha Nullah (contaminated sites) as compared to the PAU (reference site). Levels of heavy metals in the earthworm tissues were found to be higher than the soil samples. Among the three observed species of earthworms, *L. mauritii* showed higher accumulation of heavy metals, as bioconcentration factor was found to be more than that of other two species (*M. posthuma* and *M. peguana*). Furthermore, the availability of metals in soils was influenced by the soil physico-chemical properties accounting for the variation in metal concentrations from one site to the other.

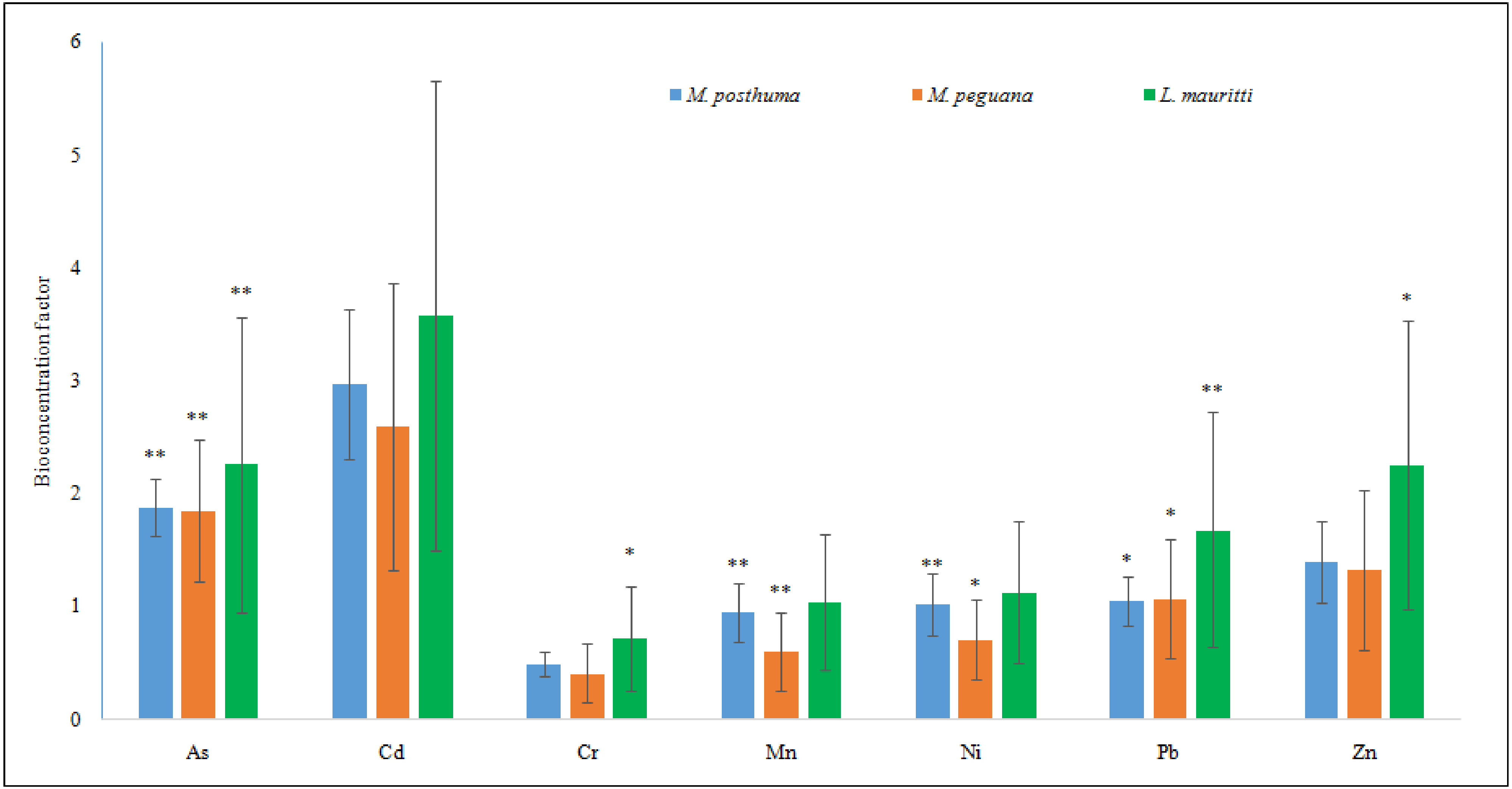

Fig. 1: Comparative analysis of bioconcentration factor for accumulation of heavy metals in different species of earthworms at selected vegetable field areas near Buddha Nullah

*Values are significant at 5% level of significance

**Values are significant at 1% level of significance

Table 1: Comparative account of different soil parameters at selected vegetable field areas in vicinity of Buddha Nullah and PAU (reference site)

Sites	Name of Village /location	pH	Electrical conductivity (dSm^{-1})	Organic matter (%)	Calcium carbonate (%)
I	Dairian	7.97±0.09	0.25±0.04	1.31±0.12	3.30±0.21
II	Jassian	8.27±0.15	0.20±0.01	0.99±0.04	3.97±0.48
III	Choorpur	7.90±0.06	0.48±0.22	1.32±0.13	5.23±0.58
IV	Balloke	8.30±0.06	0.25±0.04	1.06±0.06	3.37±0.58
Average of Buddha Nullah sites		8.10±0.11	0.30±0.06	1.17±0.10	3.97±0.45
V	PAU (reference site)	8.37±0.15	0.11±0.01	1.41±0.04	1.13±0.24
t-test value		0.453	0.492	9.035*	11.875*

Values are Mean±S.E

*Values are significant at 5% level of significance

Table 2: Total heavy metals concentration (mg/kg) in soils at selected vegetable field areas in vicinity of Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Concentration of total heavy metals in soils (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian	5.14±0.59	0.68±0.01	46.26±1.07	278.72±15.48	18.23±0.86	17.66±1.06	70.36±3.36
II	Jassian	5.95±0.32	0.75±0.01	54.46±2.27	301.25±7.39	19.98±1.09	20.70±0.67	75.09±2.38
III	Choorpur	7.05±0.29	0.82±0.01	56.78±2.07	305.33±10.34	20.95±0.78	20.39±1.46	81.32±7.23
IV	Balloke	6.34±0.67	0.81±0.01	47.75±3.59	284.03±16.18	17.91±0.83	18.37±1.72	66.85±7.64
Average of Buddha Nullah sites		6.12±0.40	0.77±0.03**	51.31±2.55	292.33±6.47	19.27±0.72	19.28±0.75	73.41±3.13
V	PAU (reference site)	3.25±0.13	0.50±0.07	31.24±2.81	168.21±11.15	12.10±1.02	10.42±1.14	37.54±3.42
t-test Value		10.155*	7.292*	10.235*	14.989*	10.964*	10.087*	10.926*
SEPA limits		20-50	0.6	250	1500-3000	60	350	300

Values are Mean±S.E
*Values are significant at 5% level of significance
**Values are above than the permissible level
Source: Kebata-Pendias (1984) and SEPA (1995)

Table 3: Heavy metals concentration (mg/kg) in tissues of *M. posthuma* collected from vegetable field areas in vicinity of Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Concentration of heavy metals in tissues (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian Village	7.46±0.11	1.41±0.23	12.90±0.11	115.79±0.24	8.53±0.17	11.42±0.13	53.56±0.24
II	Jassian Village	14.66±0.09	2.95±0.09	33.33±0.95	274.67±2.77	27.33±0.34	27.23±0.09	170.47±0.09
III	Choorpur Village	16.96±0.26	3.27±0.14	38.54±0.39	331.69±0.77	32.74±0.54	29.31±0.14	285.27±1.18
IV	Balloke Village	12.13±0.63	1.53±0.00	17.52±0.12	237.92±0.06	15.83±0.06	14.81±0.06	85.31±0.06
Average of Buddha Nullah Sites		12.80±2.04	2.29±0.48	25.57±6.15	240.02±45.68	21.11±5.48	20.69±4.45	148.65±51.80
V	PAU (reference site)	0.30±0.01	0.27±0.00	3.04±0.13	19.60±0.02	1.75±0.01	1.93±0.01	14.88±0.01
t-test Value		3.246**	1.908	1.684	3.450**	2.971**	2.509*	0.847

*Values are significant at 5% level of significance
**Values are significant at 1% level of significance

Table 4: Heavy metals concentration (mg/kg) in tissues of *M. peguana* collected from vegetable field areas in vicinity of Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Concentration of heavy metals in tissues (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian Village	11.31±0.07	3.10±0.77	18.06±0.20	156.35±0.15	16.58±0.07	18.99±0.07	126.27±0.13
II	Jassian Village	1.28±0.01	0.19±0.01	2.49±0.19	30.43±0.01	2.20±0.01	2.91±0.01	12.26±0.14
III	Choorpur Village	17.50±0.63	3.61±0.13	59.72±1.38	451.25±0.72	30.55±0.27	47.08±0.00	229.72±0.27
IV	Balloke Village	15.72±0.63	0.96±0.01	8.14±0.00	78.78±0.01	6.11±0.01	14.19±0.08	30.77±0.05
Average of Buddha Nullah Sites		11.45 3.63	1.97±0.82	22.10±12.95	179.20±94.32	13.86±6.34	20.79±9.39	99.76±50.01
V	PAU (reference site)	0.57±0.01	0.05±0.01	1.34±0.24	26.09±0.01	2.10±0.01	2.36±0.01	26.33±0.02
t-test Value		2.897**	1.469	1.692	3.546**	2.709*	2.497*	0.486

*Values are significant at 5% level of significance
**Values are significant at 1% level of significance

Table 5: Heavy metals concentration (mg/kg) in tissues of *L. mauritti* collected from vegetable field areas in vicinity of Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Concentration of heavy metals in tissues (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian Village	24.90±0.20	4.41±0.40	45.58±0.20	429.11±0.20	32.73±0.20	67.26±0.53	290.36±0.34
II	Jassian Village	1.49±0.03	0.40±0.11	8.35±0.22	104.33±0.39	7.61±0.02	6.43±0.03	40.42±0.04
III	Choorpur Village	15.72±0.78	5.64±0.12	99.103±0.12	682.94±0.33	48.07±0.22	51.54±0.38	341.66±0.25
IV	Balloke Village	2.84±0.71	0.31±0.02	0.68±0.13	7.20±0.01	0.65±0.02	1.39±0.05	7.79±0.00
Average of Buddha Nullah Sites		11.24±5.57	2.69±1.37	38.43±22.48	305.90±154.70	22.27±11.02	31.66±16.37	170.06±85.17
V	PAU (reference site)	0.14±0.00	0.24±0.01	0.57±0.00	5.46±0.03	0.50±0.00	1.08±0.00	3.84±0.00
t-test Value		3.547**	1.326	2.468*	0.849	1.782	3.976**	2.589*

*Values are significant at 5% level of significance

**Values are significant at 1% level of significance

Table 6: Bioconcentration factor (BCF) for heavy metal accumulation in tissues of *M. posthuma* collected from contaminated sites near Buddha Nullah and PAU (reference site)

`Sites	Name of village /location	Bioconcentration factor of tissue heavy metals (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian	1.45±0.02	2.08±0.34	0.28±0.00	0.42±0.00	0.47±0.01	0.65±0.00	0.76±0.00
II	Jassian	2.46±0.02	3.94±0.13	0.61±0.02	1.47±0.01	1.61±0.00	1.32±0.00	2.27±0.00
III	Choorpur	1.71±0,04	3.99±0.18	0.68±0.01	1.09±0.00	1.13±0.01	1.44±0.01	1.28±0.00
IV	Balloke	1.91±0.10	1.89±0.00	0.37±0.00	0.84±0.00	0.88±0.00	0.81±0.00	1.28±0.00
Average of Buddha Nullah Sites		1.88±0.25	2.97±0.66	0.49±0.11	0.95±0.26	1.02±0.27	1.05±0.22	1.40±0.36
V	PAU (reference site)	0.09±0.00	0.56±0.00	0.10±0.00	0.12±0.00	0.15±0.00	0.19±0.00	0.40±0.00
t-test Value		4.478**	1.477	1.478	5.590**	4.689**	2.640*	0.590

*Values are significant at 5% level of significance
**Values are significant at 1% level of significance

Table 7: Bioconcentration factor (BCF) for heavy metal accumulation in tissues of *M. peguana* collected from contaminated sites near Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Bioconcentration factor of tissue heavy metals (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian	2.20±0.02	4.56±1.14	0.39±0.00	0.56±0.00	0.91±0.00	1.08±0.00	1.79±0.00
II	Jassian	0.22±0.00	0.26±0.03	0.05±0.00	0.10±0.00	0.11±0.00	0.14±0.00	0.18±0.00
III	Choorpur	2.48±0.09	4.40±0.17	1.05±0.02	1.48±0.00	1.46±0.01	2.31±0.00	2.82±0.00
IV	Balloke	2.48±0.12	1.19±0.02	0.17±0.00	0.28±0.00	0.34±0.00	0.77±0.00	0.46±0.00
Average of Buddha Nullah Sites		1.85±0.63	2.60±1.27	0.41±0.26	0.60±0.35	0.71±0.35	1.07±0.53	1.32±0.71
V	PAU (reference site)	0.18±0.00	0.11±0.03	0.04±0.01	0.16±0.00	0.21±0.00	0.23±0.00	0.33±0.00
t-test Value		3.775**	1.381	1.464	5.659**	2.649*	2.511*	0.452

*Values are significant at 5% level of significance
**Values are significant at 1% level of significance

Table 8: Bioconcentration factor (BCF) for heavy metal accumulation in tissues of *L. mauritii* collected from contaminated sites near Buddha Nullah and PAU (reference site)

Sites	Name of village /location	Bioconcentration factor of tissue heavy metals (mg/kg)						
		As	Cd	Cr	Mn	Ni	Pb	Zn
I	Dairian	4.84±0.04	6.50±0.59	0.99±0.00	1.54±0.00	1.80±0.01	3.81±0.03	4.13±0.00
II	Jassian	0.25±0.01	0.54±0.16	0.15±0.00	0.35±0.00	0.38±0.00	0.31±0.00	0.54±0.00
III	Choorpur	3.39±0.05	6.88±0.16	1.75±0.00	2.24±0.00	2.29±0.01	2.53±0.02	4.20±0.00
IV	Balloke	0.45±0.11	0.39±0.03	0.01±0.00	0.03±0.00	0.04±0.00	0.08±0.00	0.12±0.00
Average of Buddha Nullah Sites		2.26±1.31	3.58±2.08	0.72±0.46	1.04±0.60	1.13±0.63	1.68±1.04	2.25±1.28
V	PAU (reference site)	0.04±0.00	0.50±0.02	0.02±0.00	0.03±0.00	0.04±0.00	0.10±0.00	0.10±0.00
t-test Value		3.709**	1.269	2.597*	0.658	1.723	3.741**	2.642*

*Values are significant at 5% level of significance
**Values are significant at 1% level of significance

Acknowledgements:-
Authors are thankful to Dr. Harjit Kaur, retired Professor of Zoology, PAU, for planning the work, and Head, Department of Zoology for providing necessary facilities to do this research work.

References:-
Anderson, C. and Laursen, J. (1982) Distribution of heavy metals in *Lumbricus terrestris, Aporrectode longe* and *A. rosea* measured by atomic absorption and X-ray fluorescence spectrometry. Pedobiol., 24: 347 – 356.

Anonymous (2008) *Buddha Nullah the toxic vein of Malwa/312236*. http://www.expressindia.com. Indian Express: May 21, 2008.

Awashthi, S. K. (2000) Prevention of food adulteration Act no 37 of 1954. Central and State rules as amended for 1999, 3rd edn, Ashoka Law House, New Delhi.

Ayeni, O. O., Ndakidemi, P. A., Synman, R. G. and Odendaal, J. P. (2010) Metal contamination of soils collected from four different sites along the lower Diep River, Cape town, South Africa. Int. J. Phys. Sci. 5: 2045-2051.

Bustos, O. E. and Goichochea, R. I. (2002) Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian. J. Androl., 4 3: 195-199.

Cortet, J., Vauflery, A. G. D., Balaguer, N. P., Gomot, L., Texie, C. and Cluzeau, D. (1999) The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil. Biol., 35: 115-134.

Dominguez, J., Aira, M. and Gomez-Brandon, M. (2009) The role of earthworms on the decomposition of organic matter and nutrient cycling. Ecosistemas., 2: 20-31.

Hobbelen, P. H. F., Koolhaas, J. E. and Van Gestel, C. A. M. (2006) Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. Environ. Pollut., 144: 639–646.

Jackson, M. L. (1973) *Soil chemical analysis – advanced course*. A manual of methods useful for instruction and research in soil chemistry, physical chemistry, soil fertility and soil genesis. 2nd edition Madison US.

Kabata- Pendias, A. and Pendias, H. (1984) Trace elements in soils and plants, CRC Press, Boca Raton, USA.

Kaur, G. (2015) Role of earthworm *Eisenia fetida* (Savigny) in remediating heavy metal load of contamination soil. M.Sc Thesis, PAU, Ludhiana.

Kaur, K. and Sangha, G. K. (2014) Effects of metal contaminated soils on *Eisenia fetida* (Savigny) at Ludhiana (Punjab), India. J. App. Nat. Sci., 6**:** 519-523.

Koul, S., Kocher, D. K. and Hundal, S. S. (2015) Morphometric variations in earthworms inhabiting vegetable fields around Buddha Nullah, Ludhiana (Punjab) - A Preliminary study. Agri. Res. J. (accepted).

Lanno, R., Wells, J., Conder, J., Bradham, K., Bast, N., (2004) The bioavailability of chemicals in soil for earthworms. Ecotoxicol. Environ. Safety., 57:39-47.

Lukkari, T., Teno, S., Vaisanen, A. and Haimi, J. (2006) Effect of earthworms on decomposition and metal availability in contaminated soil: Microcosm studies of populations with different exposure histories. Soil. Biol. Biochem., 38: 359-370.

Morgan, E. J. and Morgan, A. J. (1993) Seasonal change in the tissue metals (Cd, Zn and Pb). Concentration in two Ecophysiolgoically dissimitar earthworms species. Pollution monitoring implication. Environ. Pollut., 82: 1-7.

Nahmani, J. and Lavelle, P. (2002) Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. Euro. J. Soil Biol., 38: 297-300.

Nazif, W., Perveen, S. and Saleem, I. (2006) Status of micronutrients in soils of district Bhimber. Azad Jammu and Kashmir. J. Agric. Biol. Sci., 1: 35-40.

Odoh, R., Agbaji, E. B., Kagbu, J. A. and Thomas, S. A. (2011) Heavy metal speciation in agricultural farmland in some selected local government areas of Benue state, Nigeria. Archives. Appl. Sci. Research., 3: 560-573.

Olayinka, O. T., Idowu, A. B., Dedeke, G. A., Akinloye, O. A., Ademolu, K. O. and Bamgbola, A. A. (2011) Earthworm as Bio-indicator of Heavy Metal Pollution around Lafarge, Wapco Cement Factory, Ewekoro, Nigeria. *Proceedings of the Environ Manag Conf,* Federal University of Agriculture, Abeokuta, Nigeria pp 489-96.

Peijnenberg, W. J. G. M., Baerselman, R., De Groot, A. C., Jager, T., Posthuma, L. and Van Veen, R. P. M. **(**1999) Relating environmental availability to bioavailability: Soil type dependent metal accumulation in the oligochaete *Eisenia andrei*. Ecotoxicol. Environ. Safety., 44: 294-310.

Puri, A. N. (1930) A new method of estimating total carbonates in soils. Pusa Bulletin, Imperial Agri. Research, New Delhi. 73: 206-207.

Rattan, R. K., Datta, S. P., Chonkar, P. K., Sribabu, K. and Singh, A. K. (2005) Long- term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater- a case study. Agric. Ecosys. Environ., 109: 310-322.

Richard, L. A. (1954) Diagnosis and improvement of saline and alkali soils. In: *Agriculture Hand Book* 60: 7-33. USDA, USA.

Rochfort, S. J., Ezernieks, V. and Yen, V. A. (2009) NMR-based metabolomics using earthworms as potential indicators for soil health. Metabolomics., 5: 95-107.

Rodriguez, J. A., Nanos, N., Gran, J. M., Gill, J. and Lopez-Arios, M. (2008) Multiscale analysis of heavy metal contents in Spanish agricultural top soils. Chemosphere., 70: 1085-1096.

Sánchez-Martín, M. J., García-Delgado, M., Lorenzo, L. F., Rodríguez-Cruz, M. S. and Arienzo, M. (2007) Heavy metals in Sewage Sludge Amended Soils Determined by Sequential Extractions as a Function of Incubation Time of Soils. Geoderma., 142: 262-273.

SEPA (1995) National Environmental Protection Agency of China, GB 15618.

Singh, S. and Kumar, M. (2006) Heavy metal load of soil, water and vegetables in peri-urban Delhi. Environ. Monit. Assess., 120: 79-91.

Spurgeon, D. J. and Hopkin, S. P. (1996) Risk assessment of the threat of secondary poisoning by metals of predators of earthworms in the vicinity of a primary smelting works. Sci. Environ., 187**:**167-183.

Suthar, S. (2007) Nutrient changes and bio-dynamics of epigeic earthworm *Perionyx excavates* (Perrier) during recycling of some agriculture wastes. Bioresour. Technol., 98: 1608-1614

Suthar, S., Singh, S. and Dhawan, S. (2008) Earthworm as bioindicators of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological categories. Ecol. Eng., 32 :99-107.

Uzoma, K. O., Iroha, A. E., Chinyere, C. G., Sunday, E. A., Kelechukwu, D. M. and Ahuwaraeze, N. L. (2013) Effects of mining effluent contaminated soil treated with fertilizers on growth parameters, chlorophyll and proximate composition of *Cucurbita pepo* vegetable. J. Biodiver. Environ. Sci., 3: 1-8.

Walkley, A. and Black, C. A. (1934) An examination of the Digtjareff method for determination of soil organic matter and a proposed modification of chromic acid titration method. Soil Sci., 37: 29-39.

Weeks, J. M., Spurgeon, D. J., Svendsen, C., Hankard, P. K., Kammenga, J. K., Dallinger, R., Kohler, H. R., Simonsen, V. and Scott-Fordsmand, N. (2004) Critical analysis of soil invertebrate biomarkers: a field case study in Avonmouth, UK. Ecotoxicol., 13: 819-824.

Wilson, B. and Pyatt, F. B. (2007) Heavy metal dispersion ,persistence, and bioaccumulation around and ancient copper mine situated in Anglesey. UK. Ecotoxicol. Environ. Safety., 66: 224-231.