# Terrestrial Invertebrates as a Bioindicators of Heavy Metals Pollution

***Israa M. Jasim ****

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## Abstract:

Bioaccumulation of heavy metals in the terrestrial invertebrates in Al-Jadriyia district Baghdad- Iraq were investigated. Forth terrestrial invertebrates snails, slug, isopods, and diplopods , were selected for this study. The results showed that all invertebrate groups have the ability in accumulate considerable amounts of heavy metals. Higher levels of zinc and copper were observed in the isopods specimens, it's about ( 60.50±0.58 ) and ( 96.00±0.58 ) ppm respectively , while higher levels of lead were observed in the diplopods specimens ,it's about ( 23.00±1.15 ) ppm ,but the higher levels of both iron and cadmium were observed in snail specimens , it's about ( 590.00±1.15 ) and ( 9.50±1.15 ) ppm respectively .but the higher level between all heavy metals concentration in soil specimens were recorded in iron , zinc , lead, copper, and cadmium, it's about ( 8000.00±1.73 ) , ( 33.00±1.73 ) , ( 30.00±1.15 ) , ( 20.00±0.58 ) , and ( 10.00±1.73 ) ppm respectively .The correlation values show that most significant in some metals , while they show that non significant between in other metals .

**Key words: Heavy metals, terrestrial invertebrates, toxicity, bioindicators.**

## Introduction:

Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions [ 1 ] .They are considered as very important and kept under environmental pollutant category due to their highly toxic effects in the various environmental departments [ 2 ]. Inputs of metals to the environment as a result of anthropogenic activities and natural sources . And that Pb, Zn, Ni and Cu are the most important metal pollutants from human activities [ 3 ] . The heavy metals which have been studied extensively the last decades are: Cd, Hg, Zn, Cu, Ni, Cr, Pb, and Fe. Some metals that have received more attention are Hg, Cd, and Pb, because of their highly toxic properties and their effects on the environment and the living organisms[4]. Heavy metals have largest availability in soil and aquatic ecosystem and to relatively smaller proportion in atmosphere at particular vapors [2]. Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity. They reduce intake, enhance excretion of the metals within tissues to render them nontoxic. Metals can be sequestered through storage by metal binding proteins, such as metallothioneins, in cellular vesicles and granules [ 5 ]. In evaluation of pollutions in the environment the biological methods (with the use of accumulative indicators) and with the use of the organisms reacting to the changes of surrounding's chemical content are used more frequently [ 6 ] .

*Biology Department – College of Science for Women –University of Baghdad

Refer ( Marko., *etal*.2009) to the toxicity of the heavy metals such as Pb ,cd ,and Zn, and their potentially toxic for living organisms depends on their levels, chemical structures and the kinds of the organisms[7] . In pollution studies with urban organisms, invertebrates have more often been used as passive, indirect bioindicators or accumulation indicators rather than as active, direct indicators. Advantages of invertebrates for bioindication studies are the integration of pollutants in space, the short life cycle and the relative ease of collection. Some invertebrate groups like isopods, diplopods and gastropods accumulate metals to high levels, and therefore are used in applied issues as bioindicators in assessment of environmental metal pollution [ 8 ]. One important question in ecotoxicological studies refers to the choice of the bioindicator species. It will depend on its ecological and toxicological importance, facility to be maintained in laboratory, reproductive rate, sensitivity and functional importance in the ecosystem, as well as easy collection, identification and analysis [9]. The molluscs represent one of the most diverse and species-rich phyla of the animal kingdom. With more than 130,000 known recent species they are only second to the arthropods. Of the seven molluscan classes, gastropods make up more than 80% of the species ,molluscs are expanded their distribution to various terrestrial habitats ,and due to their distribution and enormous species number, molluscs play important ecological roles in the different terrestrial ecosystems of the world [10,11]. One of this roles is bioindication and biomonitoring where molluscs have been successfully used to obtain information on the quality of terrestrial ecosystems and to quantify the exposure to and effects of contaminates in their environment [12] In terrestrial ecosystems the gastropods( land snails and slugs) are the only molluscs living in it and which can be used for a bioindicators in these ecosystems [13]. Molluscs play clearly a secondary role in the surveillance of terrestrial ecosystems when compared with other invertebrate groups like nematodes, annelids and arthropods. Nevertheless, biomonitoring attempts with snails and slugs have found an increasing interest during the last decade and a number of promising projects have already been conducted. Most of them made use of the snails and slugs bioaccumulation potential for metals and organic contaminants, but there are also examples for surveys which assessed the biological effects of soil contaminants on different levels of biological organization [14] . Because some species of molluscs accumulate high amounts of metals and reflect the levels of bioavailable metals in their environment these animals are often used as quantitative indicators of metal pollution [15]. The importance of evaluating heavy metals accumulation in snails and other invertebrates to declare the important links about transferring these metals from producers to consumers (carnivores) [16 ].And due to the differences in the ecophysiology of metals, or the experimental methodology, or species difference, or food chains, so that biomagnifications of metals are not general to all environmental ecosystems [17,18].Several authors have reported the importance of molluscans as good indicators for monitoring heavy metal pollution even though the abnormally high environmental concentrations of heavy metal affects numerous biological processes involved in the development and maintenance of molluscan populations such as feeding, growth, reproduction, general physiological activities and maturity [19]. Another invertebrate group used in toxicological studies is terrestrial arthropods such as, Isopoda and Diplopoda, due to the most suitable animals to evaluate the impacts of toxic substances such as heavy metals present in the soil, because the direct contact between these arthropods and soil contaminants [ 20 , 21 ]. Terrestrial isopods are one of the largest orders of crustaceans with approximately 10.000 thousands described species, mostly bioindicators in the assessment of soil toxicity [ 22 ], and the main parameters used for estimation in toxicity analysis of soil were abundance of individuals [ 23 ] , reproduction rates [ 24 ] and organisms survival [ 25 ] . Because the isopods are accumulate high concentration of heavy metals in their digestive gland hepatopancreas they can be used as bioindicators of heavy metals ecosystems contamination [ 26 ] .With over 12 000 described species in 145 families and 16 orders [27,28], the diplopods are more component of terrestrial ecosystems, and due to their distribution in various soil layers, these animals can be much effected by the accumulation of metals and other toxic substances in the soil[29], and the most studies using these animals as bioindicators of heavy metals contamination [30].

**The aim of this study:**

The studying designed to test the efficiency of some terrestrial invertebrates groups for heavy metals accumulation and capable use it in biomonitoring these contaminants in different ecosystems.

## Materials and Methods:

**Samples collection**

Molluscs and crustacea were selected for the fauna. The molluscs being harmless were hand picked from sources, but the crustacea were caught by trapping or netting device. Upon collection, the samples were washed with tap water and transported in cellophane bags to the laboratory[ 31 ] . And they were placed in clean watch glasses and dried in an oven at 105 °C until brittle enough for grinding. After grinding to fine powder the samples were further dried to constant weight at 105 °C, and put in labeled polythene bags and preserved in a desiccators.

**Chemical analysis of organisms :**

2 g of each samples was accurately weighed into digestion flasks. A mixture of concentrated Sulfuric acid ($H_2SO_4$) and Prochloric acid ($HClO_3$) (2:1) was added and then digested. The resultant residue was dissolved in 10 ml(1:1) Hydrochloric acid (HCl); and then diluted to the 100ml mark with distilled water. This solution was used for the determination of the heavy metals in triplicates on atomic absorption spectrophotometer (AAS) in accordance with [32] and standard methods of [ 33 ].

**Chemical analysis of soil :**

1g of soil sample was accurately weighed into digestion flask after it was desiccation and grinding to fine powder . A mixture of concentrated Nitric acid ( $H_2NO_3$ ) and Hydrochloric acid ( HCl ) ( 1: 2 ) was added and then digested and diluted to the 50 ml mark with distilled waters , this solution has used for determination of heavy metals by atomic absorbance spectrophotometer(ASS) in accordance with [32,33] and the central lab of the scientific collage of Baghdad university.

**Statistical analysis**

Data were analyzed using the Statistical Analysis System ( SAS ) for study the effect of invertebrates groups in heavy metals concentrations and group means were compared using Least Significant Differences test ( LSD ) . P values < 0.01 were considered more significant and P values < 0.05 were considered significant [34].

## Results and Discussion:

Table 1 shows heavy metal concentrations in molluscs and crustacean with soil . The isopods had higher concentrations of the zinc about 60.50 ± 0.58 and then slugs, snail, and diplopods were 34.00 ± 1.73 , 29.00 ± 1.15 and 25.50 ± 1.15 ppm respectively. While the diplopods have higher concentration of the lead about 23.00 ± 1.15 and then isopods , slugs, and snails were 19.00 ± 0.29 , 17.00 ± 1.15 , and 15.00 ± 0.58ppm respectively .

While the higher concentration of copper had recorded in isopods about 96.00 ± 0.58 , and then slugs , diplopods , and snails were 70.00 ± 0.58 , 44.00 ± 0.58 , and 25.00 ± 1.73ppm respectively. While the snails had higher concentration of the iron about 590.00 ± 1.15ppm and the isopods , slugs , and diplopods were 470.00 ± 0.58 , 300.00 ± 1.15 , and 300.00 ± 1.15ppm But the cadmium had recorded higher concentration in snails about 9.50 ± 1.15ppm , and then diplopods, isopods , and slugs were 9.00 ± 1.15, 8.50 ± 0.29 , and 8.00 ± 0.58ppm.

while the soil specimens show that it have high concentration of iron about 8000.00 ± 1.73ppm and then zinc ,lead , copper , and cadmium about 33.00 ± 1.73 , 30.00 ± 1.15 , 20 .00 ± 0.58 , and 10.0 ± 1.73ppm respectively .

**Table 1. Comparison of heavy metal concentrations(Means ppm and standard deviations ) in different terrestrial invertebrate groups and soil from the area of study .**

| Metals / Specimens | Means ( ppm ) ± Standard deviations | | | | |
|---|---|---|---|---|---|
| | Zn | Pb | Cu | Fe | Cd |
| Isopoda | 60.50 ± 0.58 | 19.00 ± 0.29 | 96.00 ± 0.58 | 470.00 ± 0.58 | 8.50 ± 0.29 |
| Diplopoda | 25.50 ± 1.15 | 23.00 ± 1.15 | 44.00 ± 0.58 | 185.00 ± 1.73 | 9.00 ± 1.15 |
| Snail | 29.00 ± 1.15 | 15.00 ± 0.58 | 25.00 ± 1.73 | 590.00 ± 1.15 | 9.50 ± 1.15 |
| Slug | 34.00 ± 1.73 | 17.00 ± 1.15 | 70.00 ± 0.58 | 300.00 ± 1.15 | 8.00 ± 0.58 |
| Soil | 33.00 ± 1.73 | 30.00 ± 1.15 | 20.00 ± 0.58 | 8000.00 ± 1.73 | 10.0 ± 1.73 |
| L.S.D | 4.227* | 2.961* | 2.933* | 4.227* | 3.475 N.S |

* = $P < 0.05$ N.S = Non significant

Table 2 show the correlation factor values among all heavy metals in this study , these values were positive more significant between zinc with copper and lead with iron about 0.79 and 0.82 respectively , and between lead and iron with cadmium were positive significant correlation value about 0.44 and 0.30 respectively. But the correlation values were negative significant between lead with copper and copper with cadmium and iron about -0.38,-0.31,and -0.54 , while among zinc with cadmium , iron , and lead the correlation factor show non significant values .

**Table 2. The correlation factor values among all heavy metals concentrations in this study :**

| Heavy metals | Cd | Fe | Cu | Pb |
|---|---|---|---|---|
| Zn | - 0.03 N.S | - 0.12 N.S | 0.79 ** | 0.09 N.S |
| Pb | 0.44 * | 0.82 ** | 0.38 * | |
| Cu | - 0.31 * | - 0.54 * | | |
| Fe | 0.30 * | | | |

** = P < 0.01 * = P < 0.05
N.S = Non significant

The present study observed from the results the soil invertebrates have ability in store and accumulative variety levels of heavy metals as a result from exposure their habitats for environmental pressure especially heavy metals pollution as more common pollutants of urban region and industrial process, therefore the results of this research provide important information on condition of the environment as well as of environmental processes. There are numerous reports on the use of soil fauna, especially invertebrates as a bioaccumulation for contamination of their habitat especially heavy metals contamination , from these reports , [20] explained the metals accumulation strategies in saprophagous and phytophagous soil invertebrates quantitative comparison by exposed adult of the Isopoda , Diplopoda and Gastropoda to lead ,cadmium and zinc contaminated food and soil for three weeks. While [35] studied the bioaccumulation of heavy metals in terrestrial invertebrates were collected and compared (Arthropoda and Lumbricidea) based on total soil concentrations and body concentration. While [36] had measured the some heavy metals in alpine terrestrials invertebrates and reflect the atmospheric pollution in all ecosystems components.The terrestrial snails studied as a quantitative indicators of environmental metal pollution by (Burkhard&Reinhard,1993) where concentration of cadmium , lead , copper and zinc had measured in individuals of *Arianta arbustorum* from different urban sampling sites[15]. While the(Markert., *etal*. 2002) studied the ecological role and importance of molluscs representive one of soil organism components as a bioindicators for monitoring programs in the field[14]. But [37] studied the effect of heavy metals on survival and growth of *Biomphalaria glabrata Say* (Gastropoda: Pulmonata), and interaction with *Schistosoma* infection. But the first study carried out with diplopods, as possible bioindicators, was conducted by [38] , involving the assimilation of metals by the species *Glomeris marginata*. In this study, it was verified a higher uptake of copper, zinc and cadmium by the animals collected in soils contaminated when compared to those animals collected in non-contaminated environments. And the [39] studied distribution of elements in the millipedes *Oxidus gracilis* and relation to environmental habitats, and [40] so studied internal of millipedes *Chamberlinius hudienensis* Wang . But( Triebskorn.,*etal*.1991) exposed several invertebrates such as isopods and diplopods to different toxic substances and used the ultra structural analysis in order to demonstrate the applicability of using such animals in biomonitoring[41].

## Conclusion:

From this study the researcher concludes the ability of this groups from terrestrial invertebrates in accumulative different levels of heavy metals from their habitats and identify their role in biomonitoring and treatment ecosystems contamination .

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# اللافقريات الارضية مؤشرات حيوية للتلوث بالعناصر الثقيلة

***اسراء محسن جاسم****

*قسم علوم الحياة / كلية العلوم للبنات / جامعة بغداد

**الخلاصة :**

درس التراكم الحيوي للعناصر الثقيلة في اللافقريات الارضية في منطقة الجادرية – بغداد – العراق . اختيرت اربعة مجاميع من اللافقريالت الارضية ( القواقع ، البزاقات ، متشابهة الاقدام الارضية ، ومزدوجة الاقدام ) لهذه الدراسة .اظهرت النتائج ان مجمايع اللافقريات جميعها لها القدرة في مراكمة مقادير معينة من العناصر الثقيلة ، اذ لوحظت اعلى مستويات لعنصري الزنك والنحاس في عينات متشابهة الاقدام الارضية وبلغت ( 60.50±0.58 ) و( 96.00±0.58 ) جزء بالمليون على التوالي ، بينما لوحظ اعلى مستوى للرصاص في عينات مزدوجة الاقدام وبلغ ( 23.00±1.15 ) جزء بالمليون ، ولكن بلغت المستويات العالية لكلا العنصرين الحديد والكادميوم في عينات القواقع وكانت ( 590.00±1.15 ) و ( 9.50±1.15 ) جزء بالمليون على التوالي ، اما تراكيز العناصر الثقيلة في عينات التربة فقد سجلت اعلى مستوياتها في الحديد ، الزنك ، الرصاص ، النحاس ، والكادميوم وبلغت ( 8000.00±1.73 ) ،( 33.00±1.73 ) ،( 30.00±1.15) ، ( 20.00±0.58 ) ، و( 10.00±1.73 ) جزء بالمليون على التوالي . اظهرت قيم معامل الارتباط ان هناك علاقة معنوية عالية بين بعض العناصر ، بينما اظهرت من جانب اخر علاقة عير معنوية فيما بين بعض العناصر الاخرى .

كلمات مفتاحية : العناصر الثقيلة ، لافقريات اليابسة ، السمية ، الدلائل الحيوية .