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Heavy metal (PB) bioaccumulation study in *Eisenia fetida* and in the larvae of *Anopheles gambiae* complex using *in silico* drug docking protocols

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Abstract
Heavy metal bioaccumulation is the process by which the application of *Eisenia fetida* earthworm species in bioremediation of contaminated soil results in a decrease in the concentration of heavy metals. However, it negatively impacts earthworms and hence detrimental to agriculture. On the other hand, in adult mosquitoes, exposure to metal pollution during the larval stage significantly impacted their subsequent insecticide tolerance. The current *in silico* study, we carry out a comparative study on the effect of Heavy metal ($PbSO_4$) bioaccumulation in *Eisenia fetida* and in the larvae of *Anopheles gambiae* complex. Metallothionein protein is selected as a common biomarker for the expression of metal accumulation in both species. In order to observe the accumulation of Lead II sulphate in the Metallothionein protein of *Eisenia fetida* and in the mosquito larvae, this study's methodology entails molecular drug docking and H-bond interaction studies using sophisticated automated drug docking servers. The findings unequivocally demonstrate that amino acid residues found in *Eisenia fetida* and in the larvae of the *An. gambiae* complex bind with different heavy metals, most notably Lead II sulphate. Our findings also line up with the previously validated wet lab findings. Finally, it was determined that Lead II sulphate directly binds to Metallothionein amino acid positions of both species. Based on the expression of Metallothionein protein, these results have demonstrated that Eisenia fetida and the larvae of the *An. gambiae* complex are capable of accumulating Lead II sulphate in their body. The accumulation of metals in a specific species of earthworm and mosquito larvae, which has detrimental effects on the species' biological systems at high concentrations, is clearly described throughout this entire *In silico* study.

Keywords: Lead II sulfate, *Eisenia fetida*, larvae of *An. gambiae* complex, docking

1. Introduction

Heavy metal pollution is currently a major problem in many parts of the world [1, 2]. Chemically, heavy metal elements, such as mercury (Hg), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), arsenic (As), chromium (Cr), and zinc (Zn), have an atomic mass greater than 20 and a gravity greater than 5 g•cm−3 [3, 4, 5]. Because of its similar chemical properties and environmental response, metalloid arsenic is often classified as a heavy metal [7, 8]. Heavy metal contamination of agricultural soil has been a major concern in China ever since industrialization and technological advancement began to take hold [9]. China has serious soil metal pollution, according to the State Environmental Protection Administration [10]. Among all the contaminants, environmental chemists have focused particularly on heavy metals because of their toxicity and persistence.

Toxic hazardous materials have been polluting soil all over the world for the past few decades, which is a major cause for concern [11, 12]. Hard to decompose, soil hazard elements can find their way into water and food supply networks, posing a long-term threat to human health and food safety [13, 14]. The United States Environmental Protection Agency (USEPA) has designated heavy metals as priority control contaminants, and because of their potentially dangerous, chronic, and irreversible characteristics, they are receiving more and more attention globally [15, 16]. Although too much copper is harmful to the body, it is a necessary trace element [17].

Pb is the second most common heavy metal in the environment, after Cd, according to two recent surveys conducted by Chinese government ministries [18]. According to data from China's prestigious National Soil Pollution Investigation, 1.50 percent of soil samples had Pb contamination from 2005 to 2013 [19, 20]. Humans are primarily exposed to lead through hand-to-mouth contact with dust and soil, especially in young children. [21] Blood lead poisoning is a serious public health issue, particularly in developing countries [22]. Children that reside close to the smelters have elevated blood lead levels (BLLs) [23].

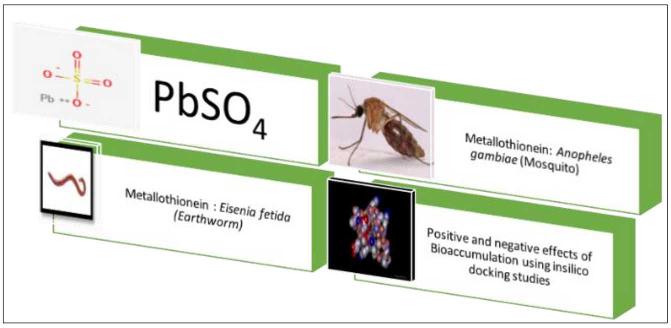
Our attention is directed towards the build-up of Lead II sulphate (PbSO4) in *Eisenia fetida* via soil and in the larvae of *Anopheles gambiae* complex. We determine how Lead II sulphate inhibits the expression of the protein, Metallothionein through Molecular Drug Docking studies. Waste from industry, agriculture, and cities has been successfully disposed of using the main earthworm species: *Eisenia foetida*, E. *andrei*, E. *eugeniae*, and *Perionyx excavates*. [24] *Erythra fetida* is widely used in earthworm composting due to its ease of cultivation. Information regarding its biological traits is abundant. *E. fetida* is capable of producing earthworm biomass and is also capable of decomposing organic waste and releasing wormcast [25].

Anopheles gambiae species complex members generally favour breeding in open, sunny, temporary, mostly unpolluted bodies of water [26]. As a result, information about members of this complex reproducing in contaminated water indicates a substantial biological change in this species [27, 28, 29]. Moreover, this transition has been documented in members of this species complex, such as An. *arabiensis*, in addition to An. *gambiae sensu stricto* [30, 31]. This adaptation may unintentionally alter biological traits of epidemiological significance, such as insecticide susceptibility, since non-pesticidal residues have been shown to modify detoxification enzyme capacity [32, 33, 34] and insecticide resistance phenotypes [35].

2. Methodology

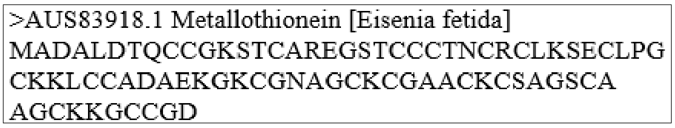
The methodology in this research study includes: 1. Target Sequence Selection. 2. Drug Docking Studies. 3. Visualization of 3D Protein – Metal Binding Interaction. (Fig :1)

1. **Target Sequence Selection:** The NCBI (National Centre for Biotechnology Information) [36] https://www.ncbi.nlm.nih.gov/ database was used to identify the potential ($PbSO_4$) metal interacting protein sequence, Metallothionein (MT_EISFE) in *Eisenia fetida* (AUS83918.1) and in (AAX86007.1) Anopheles gambiae because the database has the sequence available, based on the sequence that has been experimentally proven and the related references. [37] This sequence was mainly retrieved in order to dock it with Lead (II) Sulfate ($PbSO_4$) metal and to validate the efficiency of the interactions.
2. **Drug Docking Studies:** Numerous studies in the literature have demonstrated that lead (II) sulphate is present in soil [38]. Hence Lead (II) Sulfate (CID: 24008) was selected using NCBI PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). Metallothionein of *Eisenia fetida and Anopheles gambiae* was introduced to Lead (II) Sulfate and the metal binding to the structural domain regions of the protein sequence was viewed using HDock server [39] http://hdock.phys.hust.edu.cn/.
3. **Visualization of 3D Protein - Metal Binding Interaction:** Discovery Studio, a molecular visualisation tool, was used to validate the docking results. This software facilitates the visualisation of the intramolecular interactions between Eisenia fetida's MT protein and lead (II) sulphate and between An. gambiae's MT protein and lead (II) sulphate.



Pictorial Representation of the Research Methodology

3. Results



>AUS83918.1 Metallothionein [Eisenia fetida]
MADALDTQCCGKSTCAREGSTCCCTNCRCLKSECLPG
CKKLCCADAEKGKCGNAGCKCGAACKCSAGSCA
AGCKKGCCGD

Fig. 1: FASTA format of the Metallothionein sequence of *Eisenia fetida* and its corresponding amino acid sequence

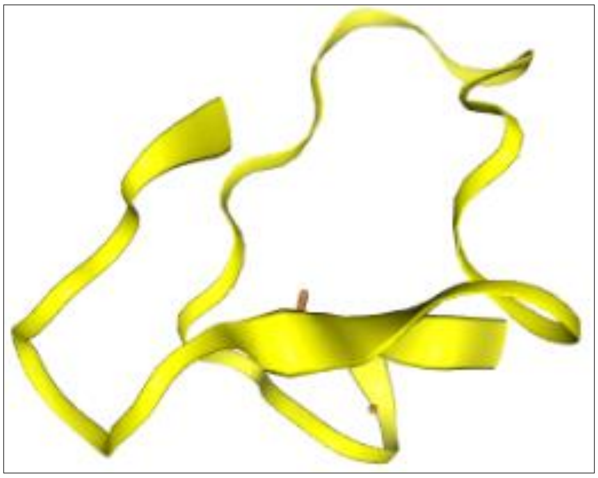


Fig 2: 3D structure of Metallothionein of *Eisenia fetida* viewed using Discovery Studio software

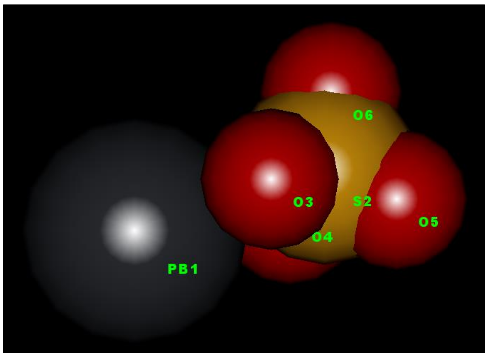


Fig 3: 3D structure of PbSO4 *viewed* using Discovery Studio software in space fill model with respective coloured atomic labels.

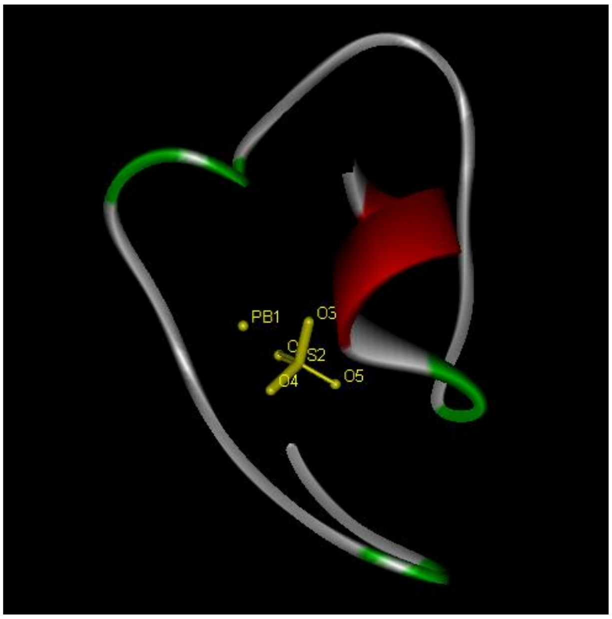


Fig 4: Complex form of Lead II sulphate and Metallothionein of *Eisenia fetida* viewed using Discovery studio software

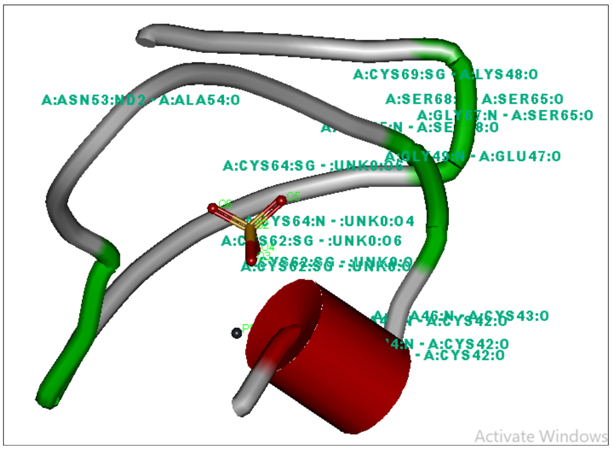


Fig 6: 3D structure complex form of Lead II sulfate and Metallothionein (*Eisenia fetida)* with respective hydrogen bond amino acid labels viewed using Discovery Studio software.

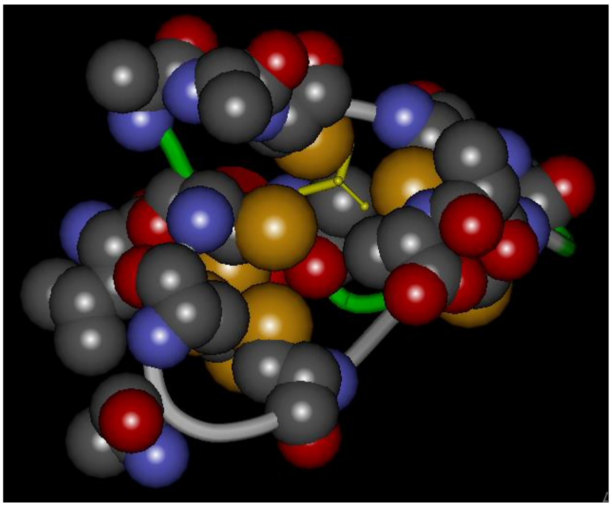


Fig 8: 3D structure complex form of Lead II sulfate and Metallothionein (*Eisenia fetida)* with respective hydrophobic interaction sites viewed using Discovery Studio software.

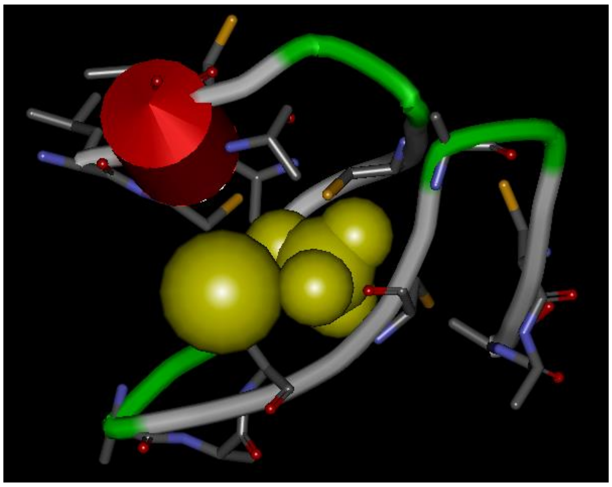


Fig 5: 3D structure complex form of Lead II sulfate (represented in yellow colour spacefill model) and Metallothionein (stick model) (*Eisenia fetida)* with respective hydrophobic interaction sites viewed using Discovery Studio software.

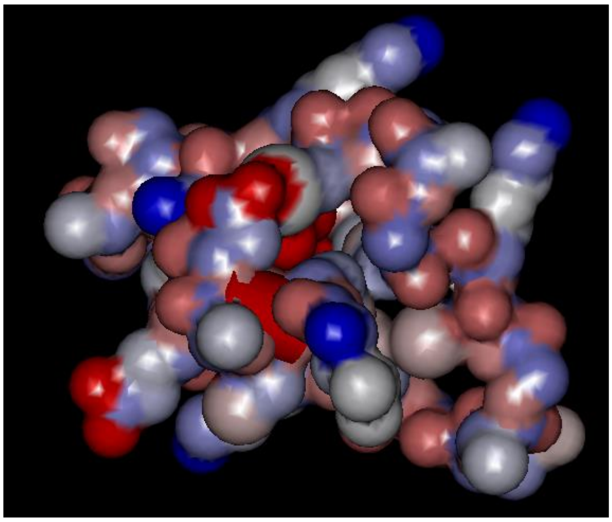


Fig 7: 3D structure complex form of Lead II sulfate and Metallothionein (*Eisenia fetida)* with electrostatic interactions between them viewed using Discovery Studio software.

>AAX86007.1 Metallothionein 2, partial [Anopheles gambiae]
MPCKTCVADCKCTSPNCGAGCGCESRCTCPCKDGAKE
GCCK

Fig: 9 FASTA format of the Metallothionein sequence of *An.Gambiae* and its corresponding amino acid sequence

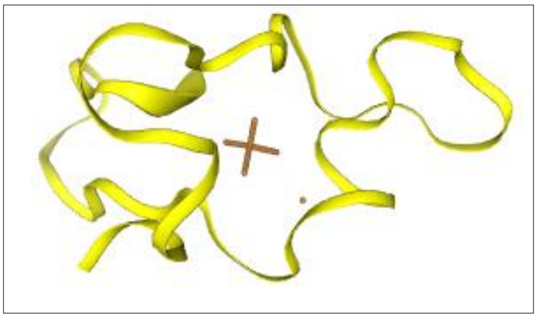


Fig 10: 3D structure of Metallothionein of An.Gambiae viewed using Discovery Studio software.

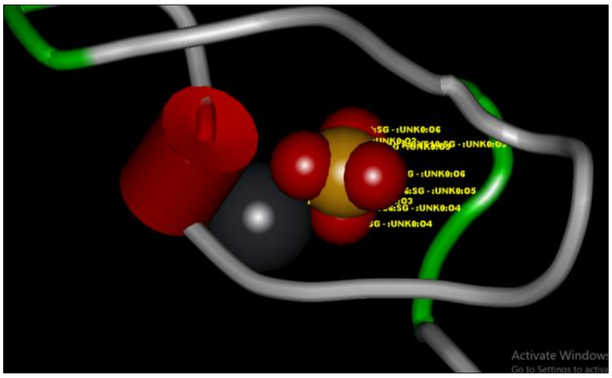


Fig 11: Complex form of Lead II sulphate and Metallothionein of An.Gambiae viewed using Discovery studio software with respective binding amino acids.

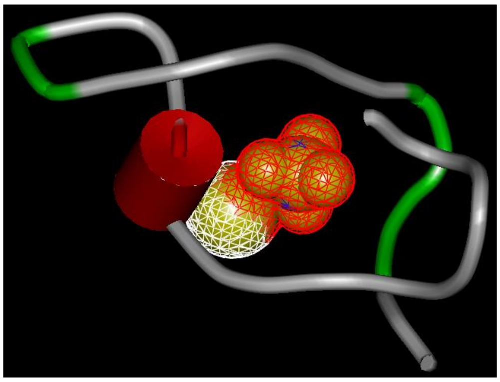


Fig 12: Complex form of Lead II sulphate and Metallothionein of An.Gambiae viewed using Discovery studio software showing 3D electrostatic interaction force

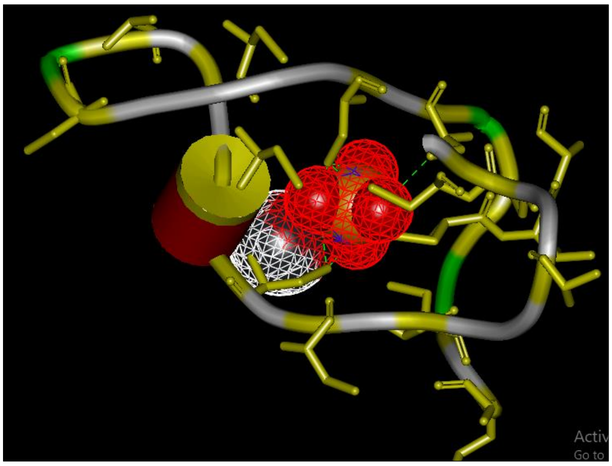


Fig 13: Complex form of Lead II sulphate and Metallothionein of An. Gambiae viewed using Discovery studio software showing 3D electrostatic - hydrophobic interaction force

Table 1: Drug docking summary

	Protein Target 1	**Protein Target 2**
Heavy Metal Compound 1	Metallothionein [*Eisenia fetida*] (AUS83918.1)	Metallothionein [Anopheles gambiae] (AAX86007.1)
Lead II Sulfate ($PbSO_4$) (CID: 24008)	-35.39 *kcal/mol.*	-41.39 *kcal/mol.*

Table 1 Molecular binding affinity scores with units between the Metallothionein *Eisenia fetida* and *Anopheles gambiae* heavy metal ($PbSO_4$)

4. Discussion

In this study, the length of the Metallothionein protein sequence of *Eisenia fetida* is 80 aa amino acids (Fig: 2, 3), and the length of the Metallothionein protein sequence of *An. Gambiae is* (41 aa) (Fig: 9, 10) that of the selected heavy metal, Lead (II) sulphate ($PbSO_4$) (Anglislite) (PubChem CID: 24008) is 303.92838 g/mol molecular weight. It contains Topological Polar Surface Area of 88.6 A^2 and a heavy atom count of 6. (Fig: 3) An important part of improving the biological, chemical, and physical characteristics of soil is done by earthworms. They serve as major bioindicators of environmental contamination and are regarded as keystone species within ecosystems.

Agriculture has been based on soil for ages, as soil is the most valuable natural resource and mankind's greatest legacy. As a result of human progress towards industrialization, dangerous pollutants like heavy metals, toxins, and carcinogenic compounds are produced and released into the environment. It is well known that earthworms have positive effects on plant growth, nutrient cycling, and soil fertility. By burrowing and casting, earthworm activity improves the physical conditions of the soil by forming stabilised aggregates that facilitate easy air and water penetration. Because of their toxicity and tendency to accumulate in the environment, heavy metals are the primary environmental pollutants and a major concern. Heavy metal-contaminated soils are one of the environmental problems that are thought to pose a major risk to the health of humans and other living things [40].

Heavy metal pollution is one of the most serious environmental issues of all types of pollution. Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) are the main heavy metal pollutants found in the environment [41, 42].

One of the main things influencing the amount of heavy metals in the soil is the use of fertilisers. The addition of mineral additives to animal feed is another factor. Livestock requires minerals like Cu, Zn, Fe, Cr, Mn, and Co. Livestock are exposed to non-essential trace elements like cadmium, mercury, arsenic, lead, and other heavy metals because mineral additives used in animal feed have low purity. The majority of heavy metals in feed are excreted by animals and used as "farmyard manure," which modifies the concentration of heavy metals in soil used on farms. Only trace amounts of these metals are absorbed by animals [60]. Earthworms in the soil absorb these heavy metals.

The Important factors influencing the resistance phenotype in urban environments has been identified as metal pollution. The larvae pay a price in fitness when they have to adapt to the presence of metal pollutants in the environment. Under laboratory conditions, An. gambiae can be selected for metal tolerance quite quickly [43]. Nevertheless, there are substantial biological costs associated with this adaptation, such as decreased egg viability, immature survivorship and emergence, and decreased reproductive capacity [44].

4.1 *In silico* docking of Metal – Protein Docking

In this docking study, HDOCK server was applied to dock the Metallothionein protein sequence of *Eisenia fetida* with Lead II sulfate. (Fig: 4). The application of HDOCK server to dock the lead II sulfate-containing Metallothionein protein sequence of Eisenia fetida is demonstrated in Figure 5. The server uses a hybrid algorithm that combines template-free and template-based docking to automatically predict the interaction between receptor and ligand molecules when data about them is entered. Unlike other similar docking servers, the HDOCK server can accept amino acid sequences as input. It also uses a hybrid docking strategy that allows experimental data about small-angle X-ray scattering and protein–protein binding sites to be included during the docking and post-docking phases [45, 46, 47, 48, 49, 50]. Table 1 shows the docking score between $PbSO_4$ and Metallothionein of *Eisenia fetida* which is -35.39 *kcal/mol.* Table 1 shows the docking score of -41.39 *kcal/mol* between $PbSO_4$ and Metallothionein of *An. gambiae.*

Figures 4, 5 clearly illustrates the molecular dynamics of how Metallothionein of *Eisenia fetida* binds with Lead II sulfate at various interacting sites. Fig.6,7,8 clearly represents the hydrophobic and electrostatic interaction between the metal and the protein of *Eisenia fetida*, viewed using Discovery Studio software. Figures: 11, 12 clearly illustrates the molecular dynamics of how Metallothionein of *An. gambiae* binds with Lead II sulfate at various interacting sites. Using Discovery Studio software, Fig: 13 depicts the hydrophobic and electrostatic interaction between the metal and the *An. gambiae*'s MT protein. The results of previous docking studies conducted in literature coincide with our findings [51, 52, 53, 54, 55, 56].

The results clearly show that the amino acids, (ALA:44,ASP:45,ALA:46,GLY:49,ASN:53,CYS:62,CYC:62 ,CYS:64,CYS:64,SER:65,GLY:67,SER:65,GLY:67,SER:68, CYS:69), are in charge of lead II sulphate ($PbSO_4$) build-up inside the earthworm's body. 9-78 CYS_RICH (PS50311): Drug binding Domain: 72-77 (GLY: 77) and 52-57 (ASN: 53). The results clearly show that the amino acids, CYS:65,CYS:6,CYS:6,CYS:10,CYS:12,CYS:12,CYS:27,CYS:27, are in charge of lead II sulphate ($PbSO_4$) build-up inside the earthworm's body. (PROSITE: PS51257: Prokaryotic membrane lipoprotein lipid attachment site profile.) 1-23 is the motif range. The drug binding sites are present in the motif regions of MT of An. *gambiae*.

Previous research in the literature has demonstrated that the high cysteine residue content of the Metallothioneins found in Eisenia fetida allows them to bind with a variety of heavy metals, most notably Lead II sulphate [57, 58]. Furthermore, it has been demonstrated in wet lab studies that Eisenia fetida's accumulation of lead II sulfate causes the protein Metallothionein to be expressed [59].

These findings are consistent with our ongoing in silico study, which has also demonstrated how the accumulation of the heavy metal, Lead II sulphate in *Eisenia fetida* and *An. gambiae* takes place and how it is expressed through the protein, Metallothionein.

5. Conclusion

Our current *In silico* research investigation primarily focuses on the molecular interactions between lead II sulphate and the *Eisenia fetida* protein and the larvae of An.gambiae using state-of-the-art *In silico* study protocols. The overall findings of our study indicate that the expression of the Metallothionein protein of *Eisenia fetida* and larval An.gambiae is based on the accumulation of lead II sulphate metal. Ultimately, our current research has revealed that the binding of the metal Lead II sulphate with the two species results in adverse effects on both species, but in the case of mosquitoes, it benefits the human population, and in the case of An. gambiae larvae, it has an adverse effect on agriculture.

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