Peertechz

Open Journal of Environmental Biology
mail.biology@peertechz.com
LIFE SCIENCES GROUP

OPEN JOURNAL OF Environmental Biology

ISSN: 2690-0777 DOI: https://dx.doi.org/10.17352/ojeb

Review Article

Metallothioneins in Earthworms: The Journey So Far

Ogunlaja Aemere[1,2]*, Vikas Sharma[2] and Johnson Lin[2]

[1]Department of Biological Sciences, Redeemer's University, Ede Osun state, Nigeria

[2]School of Life Sciences, Discipline of Microbiology, University of KwaZulu-Natal, Durban, South Africa

Received: 07 February, 2020
Accepted: 22 October, 2020
Published: 23 October, 2020

***Corresponding author:** Ogunlaja Aemere, Department of Biological Sciences, Redeemer's University, Ede Osun state, Nigeria, Tel: +2348150664670; E-mail: aigbomere@yahoo.com

Keywords: Heavy metals; Nanoparticles; Metallothioneins; Invertebrates

https://www.peertechz.com

Check for updates

Abstract

Earthworms play important roles in terrestrial ecosystems including evaluating the health status of the soil in environmental studies. Its regulation and detoxification of metallic metals and the non-essential metal ion are associated with the possession of Metallothioneins (MTs). Three isoforms of MTs are induced in some species of earthworms under stress in the soil; wMT1, wMT2, and wMT3 (found in cocoons). Though reports on the nucleotide sequences, mechanisms of action and entire functions of two earthworm MTs isoforms exist, the precise mechanism of action and entire functions of wMT3 are still obscure. Metals and stress are known inducers of MTs in earthworms. In recent times, Contaminants of Emerging Concerns (CECs) and the advent of nanotechnology has occasioned a handful of studies evaluating their effect in the environment using biomarkers like metallothioneins. More research focusing on CECs' and nanoparticles' ecotoxicological impact in the environment by monitoring biomarkers like earthworm metallothioneins is encouraged. The detection and quantification of MTs involve a wide array of techniques including analytical, instrumentation and molecular analyses which remains the most commonly used. This review evaluates the various methods and highlights their pros and cons.

Introduction

The soil is a major repository for contamination where terrestrial organisms are exposed to pollution. Earthworms are important organism in the terrestrial ecosystem and their ecological functions are indispensable as they participate in various processes in soil. They form significant biomass in the terrestrial ecosystem and they occupy a sensitive position in the food chain. *Lumbricus terrestris, L. rubellus, Eisenia fetida and E. andrei* are relevant earthworm species for monitoring environmental pollution [1] in terrestrial ecotoxicology studies. This is attributed to their capability to accumulate and tolerate elevated amounts of toxic metals within a certain threshold without experiencing significant damages [2]. Their survival and tolerance are dependent on the regulation/excretion of metallic trace elements and detoxification of non-essential toxic metal ions.

Earthworms are affected by soil contaminants at the various levels of biological organization from sub-organismal, individual to population levels. The passageways of contact with contaminants are majorly through the skin from the interstitial pore or from the ingestion of soil particles into their guts [3]. In their adaptive responses to such environmental stress, they exhibit non-transferable physiological adaptations which could induce metabolic modifications making them more tolerable to such environmental changes [4] like metal contamination [5] and [6]. On the other hand, the coping mechanisms could involve changes that would be transferable to offspring hence forming ecotypes of earthworm species based on location found [7].

There are standardized protocols for earthworm acute and sublethal testings of chemicals in contaminated soils [1] based on their responses and behavioural patterns [9]. Advances in molecular biology make use of biomarkers as rapid diagnostic and predictive tools in environmental assessments [10]. The use of genetic biomarkers gives better insight into ecotoxicological assessments as gene expression underscores changes in functionality at all levels of organizations and the predictive effect on the ecosystem. A protocol developed from a target gene can be extrapolated and used for similar genes in other related organisms [11]; hence this approach is more reliable than conventional earthworm testings [12]. Molecular markers are generally used because they typically indicate the susceptibility of organisms to contaminants or stressors. The molecular biomarkers monitored in earthworm ecotoxicological studies include Carboxylesterase (CES), Acetylcholinesterase (AChE), Catalase (CAT) and Glutathione S Transferase (GST) activity, the concentration of glutathione (GSH), [13]. Other genetic markers used in such studies are metallothioneins, annetocin [14]. Their presence and levels in organisms are

Citation: Aemere O, Sharma V, Lin J (2020) Metallothioneins in Earthworms: The Journey So Far. Open J Environ Biol 5(1): 014-021.
DOI: https://dx.doi.org/10.17352/ojeb.000016

indicative of tolerance to metal, stress and other physiological forms of pollution hence their suitability as biomarkers and indicators of environmental status and pollution.

Metallothioneins (MTs) are genetic biomarkers commonly monitored in annelids as it is referred to as the best-known biomarker candidate among Oligochaeta Annelida [15]. The common earthworm MT isoforms reported in works of literature are wMT$_1$ and wMT$_2$ [16], their induction when exposed to stress and contaminants, mechanisms of action in response to metals and their affinity to metals are reasonably investigated. However, a third isoform is wMT^3 detected at the embryonic stage of earthworms [17]; except this report, there is no other known report on wMT^3. Its structure and distinct mechanism of action remain obscure. This works compares the roles of the wMTs along with their mechanisms of action as well as highlighting significant milestones in the progressive investigation of earthworm MTs. This work also centers on the detection of MTs in earthworms and their limitations with emphasis on the new technologies. We also reviewed the reports on and highlighted pitfalls in environmental monitoring of metallothioneins in earthworms exposed to Contaminants of Emerging Concerns (CECs) and crises of new technologies like nanotechnology on earthworm MTs.

Metallothioneins

The first metallothionein was identified by Margoshes and Vallee [18] and a myriad of research followed with a focus on vertebrate and mammalian isoforms [19] and [20]. Their roles in the medical field are well reported [21] and [22]. Since its first report, more than 11500 articles on metallothioneins are cited in PubMed, and about one-tenth of these are related to environmental studies. MTs are low molecular weight cysteine-rich (up to 33% by composition) ubiquitous proteins expressed by organisms under stress condition especially when induced by metals at certain levels, making them very well-studied targets. They are heat-stable [23] and have approximately 70 amino acids [24,25]. MTs are encoded by a multigene family which vary in their responses to different inducers including heavy metals, glucocorticoids, hormones, oxidants, strenuous exercise, superoxide and hydroxyl radicals generated by gamma radiation and cold exposure [26]. The major roles of MTs include the homeostasis of trace metals (Zn, Cu, Mn, Fe etc), protection against oxidative stress and detoxification of xenobiotic metals (Pd, Cd etc) [27] and [28], metal ion transport, maintaining redox pool, scavenging of radicals and regulation of expression as explained and depicted in Figure 1 [29]. They are found in a range of organisms from microbes to mammals and reports on invertebrate MTs include nematodes [30], annelids [31]; insects [32]; the oysters [33] and various species of gastropods [34,35].

MTs have shown functional variability among organisms and significant sequence heterogeneity [36] between taxa but notable conserved regions within phylogenetically related taxa [30]. Extensive reports on their detection, roles, mechanisms of action and stoichiometry in a wide variety of organisms [37] are available.

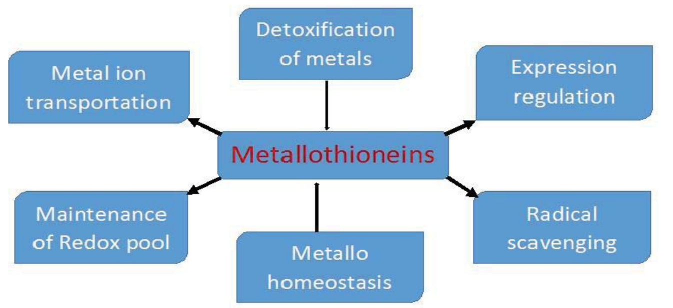

Figure 1: Main functions of metallothionein in an organism. MT participates mainly in maintaining of metal ions homoestasis, toxic metal ions detoxication, metal ions transport, maintaining redox pool, scavenging of radicals and regulation of expression. Adapted from Ryvolova, et al. 2011 [29] with slight modification.

The basic structure of metallothioneins

The structures of proteins depict their functionalities. Metallothionein has a chemical configuration often occurring as a straight polypeptide chain of cysteine (cys-cys) or cysteine having other amino acids within the chain (cys – x - cys) [38]. This makes it form better binding cluster since cysteine possesses the thiolate – SH end for metal attachment [39]. Individual cysteine residue required for metal ion binding is typically insufficient, hence the cluster forming tetrahedral binding arrangement using bridging sulphur binding ligands. The sulphur groups of cysteines are usually positioned adjacent to themselves hence encouraging the clustering.

Their chemical configurations of various families of MTs are reported but the 3D depictions are scarce [40]. Although there are structural diversity among MTs in organisms, the functional domains (C- and N-) for metal binding is usually common, appearing as "dumbbell". The functional domains only form 3D structures upon metal coordination, and when there are no metal ions, (apo-thionein or apo-T), the domains usually appear unstructured; their structure depicts their functionality [41]. One elucidated Mt 3D structure is the mammalian MTs; they have two metal-binding domains that form metal-cysteine clusters at the N- and C terminals [42]. They have structures configured to form folded metal-binding domains with the α-domain closer to C-terminal and more stable while the other is a more reactive β-domain, which is closer to N-terminal. The metal clusters formed are named "M4Cys11 (α-domain) and M3Cys9 (β-domain)" where M represents a divalent metal ion like Zn2+ or Cd2+ [43]. The functional domains are linked with varying lengths of amino acid sequences; these linkers determine the structural stability of the MT.

Earthworm metallothioneins structure

The mechanisms of tolerance of earthworms to metal by accumulation are attributed to expression of MTs and their formation of metal-rich granules (MRGs) [44]. Metal toxicity will only occur when the capacity of these mechanisms to bind metals is exceeded [45]. Unlike vertebrate MTs where similarities occur structurally, invertebrates MTs show inter / intra – structural diversities hence they have distant phylogenic relationships. This diversity could be due to their evolutionary changes in adaptation to their environment, which constantly predisposes them to contaminants.

MTs do exist in homologues and are referred to as isoforms in the literature [46]; invertebrates like snails and earthworms have eight and three MT isoforms respectively. The most-reported earthworm MT isoform are wMT1 and wMT2. They are often described to have a reverse mammalian MT arrangement "C- M4Cys11, α-domain and N- M3Cys9, β-domain" just as most vertebrates. Instead, there is an "N-terminal α-domain (M4Cys11-cluster); C-terminal β-domain (M3Cys9-cluster)" [42] arrangement depicted in Figure 2. These two isoforms (wMT1 and wMT2) have greater than 75% similarities in their sequences but differ considerably in the length and composition of their linker sequences. wMT1 have longer linker regions (6 residues), and it is less stable than wMT2 with shorter linker sequences (4 residues) wMT2 has shown more stability in its metal retention with a wider range of pH and its effectiveness in cadmium toxicity protection than wMT1 [47].

Induction of metallothioneins in earthworms

Ecotoxicology studies involving earthworms earlier attributed major forms of cellular management of excess heavy metal to the possession of chloragosomes [48,49]. Figure 3 depicts a conceptual model of impacts on soil metal chemistry due to exposure of earthworms to metal contaminated soils.

One of the tolerance mechanisms of genetic origin is the induction of metallothionein and it is reported in several earthworm species. They include *E. fetida* [50,51], *E. Andrei*, [52,53] and *Libyodrilus violaceous* [54].The genetic origin of resistance is attributed to evolutionary changes in MT gene and researches suggest that MTs are the basis of metal resistance and tolerance in these organisms [55]. Earthworm MTs mainly function in metal detoxification and evidence indicate that. Studies had shown metallothionein induction and their regulation in insects and vertebrates were conserved [57,58], it involved the binding of metal transcription factor 1 (MTF-1) to metal responsive elements (MREs) usually found in the MT genes promoter. It was however established that the transcriptional activation of MTs in invertebrate is not consistent with that of the insects and invertebrates [59] but the exact mechanism is unclear. Instead, MREs were found in the invertebrate MT gene promoters in *Lumbricus rubellus* [60] and cAMP responsive element (CRE) was found to be involved in Cd-induced Wmt2 transcription and acted as a transcriptional activator of invertebrate MTs. Metallothionein as biomarker are monitored in earthworms for Cd contamination [61,62] and other metals like mercury and $CuSO_4$ [63,64] and metallothionein monitoring in earthworm ecotoxicological studies is common.

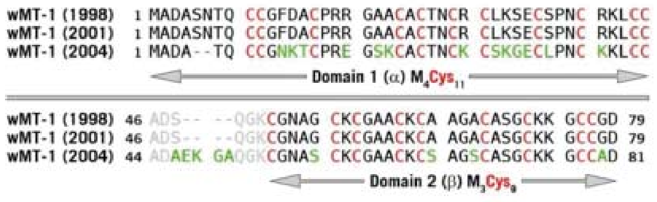

Figure 2: Aligned sequences of wMT1 from reports publishing wMT1 sequence showing proposed two domain structures with "N-terminal alpha-domain (M4Cys11-cluster); C-terminal beta-domain (M3Cys9-cluster)" arrangement. Adapted from Kowald, et al. 2012 [40].

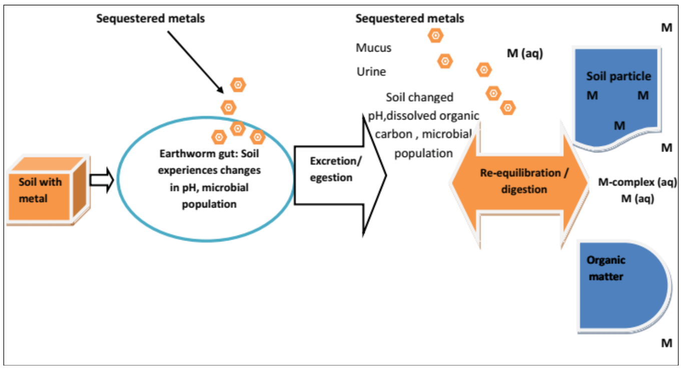

Figure 3: A conceptual model of possible impacts of earthworms on soil metal chemistry. Ingested soil travels through the gut and is egested. The egested soil may have a different pH, bacterial population and dissolved organic carbon content, all of which may modify soil chemistry. Modified bacterial populations may impact on organic matter sorbed metals. pH and dissolved organic matter changes due to egestion of soil and / or excretion of mucus and urine may impact on sorbed metals. Some metals may be sequestered in earthworm tissues and subsequently excreted in a form different from the ingested metals. Adapted from Sizmur and Hodson, 2009 [56] metal exposure correlates with production of MTs and consequently cause the reduction in metal toxicity [53].

Earthworm metallothioneins induction by metals

In earthworms, metallothionein induction of two metal responsive proteins is known. They have nucleotide and amino acid sequences similarities of 80.9% and 74.7%, respectively but a distinctive deletion/insertion of two amino acids [65]. Their coding regions show a conserved arrangement of the cysteine residues which lack aromatic amino acids. The sequences of the two isoforms (wMT1 and wMT2) are structurally similar to other invertebrate MTs. The Metallothionein gene, *Wmt2*, is known to express the most responsive protein among wMTs. wMT3 is a third isoform of earthworm metallothioneins derived from an EST library generated from developing cocoon and highly expressed in embryonic development. It is 67% similar and 56% identical with wMT1 and wMT2 however, their role remains unclear. The three wMTs isoforms differ in their expression patterns and levels when exposed to metal ions.

After the first report on earthworm wMTs, their modes of action needed further elucidation; presently, with the advent of ecotoxicogenomic approaches, a handful of such reports are available. Studies reveal that wMT1 and wMT2 bind approximately six [6] Cd2+ in two domains and the report also indicates that recombinant WMTs coordinates seven [7] Cd2+ (Cd3Cys9 and Cd4Cys11); the MT contain 20 cysteines. These MTs are like the 20-cysteine in mammals, but the overall protein structures are different being that their 11-cys and 9–cys segments are at alternate positions (i.e. the N – and C - terminus).

A study of their biological function including biophysical properties, affinities to particular metals and protein folding of wMT2 revealed there are significant differences in the

Citation: Aemere O, Sharma V, Lin J (2020) Metallothioneins in Earthworms: The Journey So Far. Open J Environ Biol 5(1): 014-021. DOI: https://dx.doi.org/10.17352/ojeb.000016

stoichiometry and protein folding of Zn-wMT2 and Cd-wMT2, conferring wMT2 its function in Cd accumulation [46]. Though, Zn (II), Cd (II), or Cu (I) are metal ions known to form metallothionein clusters, the report on their overall affinity are species dependent. The study of Foster and Robinson [66] reported HpMTs affinity as Cu(I) > Cd(II) > Zn(II) and Cu(I) being the most competitive ion. The study indicated that the selection and discrimination of metals by metallothioneins was not entirely based on overall affinity instead on the interplay of other factors. wMTs preference for metals is reported in some investigations but show inconsistences; this remains an area that require further explication.

Earthworm metallothionein induction by Contaminants of Emerging Concerns (CECs)

Substances other than metals are known contaminants found in the environment where they cause detrimental effects on the biota, among such are organic secretions like toxins and drugs, especially antibiotics; they are grouped as CECs. Investigating metallothionein induction due to these contaminants is of interest in recent times and a few reports are available. van OmmenKloeke [67] reported expression of MTs in *E. Andrei* induced by low concentrations of 2-phenylethyl-isothiocyanates (ITCs), a known natural toxin. MT was recommended as an early biomarker of ITCs contamination even at low concentrations. Colistin is a feed additive used by animal farmers as antibiotics and nutrient enhancer [68]. Its suppression of MTs is shown by Guo, et al. [69] and they indicated that colistin in soils interfered with other molecular markers in metal ecotoxicity study, but MT served as early biomarker for colistin contamination. Enrofloxacin is another antibiotic used in veterinary but did not induce MT in *E. fetida* [70]. Not all CECs are inducers of MTs. The CECs in the environment have gained attention in environmental studies because of their health implication. Their induction of molecular markers like MT indicated by few reports therefore implies that environmental monitoring with biomarkers like MT in earthworm for CECs is plausible. Elaborate investigation on other CECs as inducers of MT is therefore encouraged as only a few literature exist presently.

Earthworm metallothionein induction by nanoparticle

Nano-particles are described as substances in nanometer scales which are 1000 times smaller than normal bacteria. These nanoparticles are used in designing and manufacturing of various consumer products [71,72]. Natural nanoparticles of clay minerals, metal (hydr) oxides, humic substances are well-known examples of natural nanoparticles in soils. These nanoparticles because of their small size and large surface area have unique and novel properties. They are used in a wide variety of products from agrochemicals, food, textiles to solar panels and waste water treatment plants. The properties of nanoparticles can be further enhanced by surface coating of biocompatible molecules and stabilizing the surface, hence their surface charges, solubility and/or hydrophobicity changes depending upon the kind of biomolecule or the process of stabilization [73,74]. Global production of nanoparticles is projected to increase hence the usage and disposal of these materials will be enormous. Commonly used nanoparticles include AgNPs, CoNPsCuNPs, ZnNPs and AuNPs. Study on their environmental impact is of necessity, especially in the soil ecosystem where they are subject to transformations, aggregation/agglomeration and reaction with other biomolecules, exchange of surface elements and other redox reactions [74]. These properties make them behave differently with living organisms with respect to their parent metal.

A few nano-related ecotoxicology studies monitoring molecular markers in earthworms are available. This include assessing levels of biomarkers like Lipid Peroxidation (LPO), total, reduced and oxidized glutathione content (TG,GSH and GSSG), the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx),glutathione S-transferases (GSTs) and cholinesterases (ChEs) in *Enchytraeus albidus* exposed to ionic copper and copper nanoparticles (75). Such studies also include genotoxic (comet assay) and oxidative effects (SOD activity, TBARS) of functionalized-QDs and cadmium chloride on *Hedistedi versicolor* and *Eisenia fetida* coelomocytes [76].

Just a handful of investigations involve MTs' use as biomarkers in nano – related ecotoxicological studies with few focusing on the detection and quantification of metallothioneins in earthworms. Inductions of MT in earthworms are recorded in recent studies of Unrine, et al. [77,78]. Other such investigations include *Enchytraeus crypticus* exposed to AgNP [79], *Lumbricus rubellus* and their coelomocytes impacted by AgNPs (NM-300 K) [80] and AgNPs exposure to *E. fetida* causing transcriptional expression of MT [81]. The presence of nanoparticles, drugs and toxins in the environment and their impact are areas of interest in recent time, such studies involving earthworm MTs are under reported hence more investigations in this area are encouraged.

Methods of Metallothionein (Mts) Detection and Quantification

The earliest detection of organic substances like cystine was by Heyrousky polarography [82] while the first detection of metallothioneins was by Differential pulse polarography - DPP method [83]. In the earlier approach, cystine was the only amino acid that showed a polarographic reaction in a solution of ammonium chloride, ammonia and cobaltous chloride (Brdicka electrolyte). Conversely, cysteine and other thioacids act catalytically in the Brdcka solution which they owe to their sulfhydryl groups, the technique involves the catalysis of hydrogen in the presence of a protein containing SH- groups. Using this technique, the quantification of cysteine and others were reported by Brdicka [84,85] hence the subsequent use of the term "Bridcka reaction" by Thompson and Cosson [83]. With this method, Cystine and cysteine were quantified in pure solutions and hydrolysates of organic substances in work by Stern, et al. [82]. Several efforts have been made in the modification of DPP technique which had yielded better results like better detection limits, rapid assays, increased sensitivity etc [86].

Series of techniques including colorimetric, fractionation, paper electrophoresis etc were involved in the detection

Citation: Aemere O, Sharma V, Lin J (2020) Metallothioneins in Earthworms: The Journey So Far. Open J Environ Biol 5(1): 014-021.
DOI: https://dx.doi.org/10.17352/ojeb.000016

of the first MTs [18]. Brdicka reaction (with several modifications - AdTS, AdTS CV, AdTS DPV) was commonly used in metallothionein detection and quantification in various organisms [87,88]. Other MT detection involved using metal saturation assays in monitoring Mt in fish [89] and terrestrial organisms [90]. The method involves equating the quantity of MTs as a total saturation of their sulphydryl groups by metal ions. This estimation was misleading as other metal-binding ligands also exist in these biological systems could interfere with the estimation [91,92].

The present-day technique used in the detection and quantification of MTs range from electrochemical to bioanalytical and molecular methods. These methods involve procedures like ELISA, enzyme-linked assays, chromatography, electrophoresis, mass spectrometry, inductive coupled plasma mass spectrometry, electrochemistry, etc. Most of these techniques, however, do have their pros and cons. The immunochemical technique was the most commonly reported in publications in metallothionein detection between 2001 and 2010 [29], it is specific and sensitive however limited by the difficulty to obtain MTs antibodies among other disadvantages [93]. The electrochemical techniques like AdTS, AdTS CV, AdTS DPV [94,95] were sensitive and could detect MT peaks but require the use of analyser such as AUTOLAB Analyzer [24].

The improvement of fluorescent technique for MT detection resulted in detecting trace amount of MTs where fluorescent agents like ammonium-7fluorobenzo-2-oxa- 1, 3-diazole-4-sulfonate (SBD-F) [96] and monobromobimane (mBBr) [97] are derived. Geng, et al. [98] further improved on the fluorimetric method for MT quantification; it was sensitive to a wide range of MT concentrations and gave a relatively accurate estimation of MT. It however required tandem column system to separate the derived compound to eliminate interference or require a prior MT purification before derivation. Also, improved colorimetric method for detecting metallothioneins (MTs) was developed by Qian, et al. [99]. It involves using a thymine (T)-rich oligonucleotide (TRO)–Hg–AuNP system. The thiol groups of MTs could interact with mercury from the T–Hg2+–T complex to release TRO, resulting in a colour change of the system. MTs concentration of the range 2.56 x108 to 3.08 x 107 mol/L and the detection limit of 7.67 x 109 mol/ L were possible. This method allows direct analysis of the samples by the naked eye without costly instruments, and it is reliable, inexpensive, and sensitive.

The advent of high-performance liquid-phase chromatography-electrospray tandem mass spectrometry (HPLC ESI MS) and high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) promised more accurate quantification of metallothioneins. The high costs and technicalities of this equipment remain an imperative factor to consider in their use for the advancement of biological research. Biomolecular method, e.g. ELISA, MT - mRNA (PCR and QT-PCR) are standard method used in detecting and quantifying of metallothioneins; they are simple, less technical and accessible. They can be used to distinguish Mt-isoforms but the mRNA concentration does not give an accurate estimate of the protein concentration [93].

The first detection of earthworm MTs reported in 1998 [16] required the combination of gel chromatographic techniques and "novel" molecular methodologies (Directed Differential Display and quantitative PCR. Recent reports on earthworm MTs detection and quantification indicate molecular based kit as the most commonly used method. They are reliable but require devices like PCR and QPCR. These equipments and their consumables are relatively expensive.

Conclusion

Metallothioneins among other biomarkers impact on pollution tolerance and management in the ecosystem are well documented. Techniques involving High performance liquid-phase chromatography - electrospray tandem mass spectrometry (HPLCESI-MS), high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) are used for the detection and quantification of MT; they are expensive, requires technical – know - how and are not readily available. Other methods include flourimetric method and biomolecular methods but the biomolecular method is the most accessible and commonly in used. Earthworms play vital role in metal detoxification and maintenance and this functionality is associated with MTs. Studies have indicated that three MTs isoforms of earthworms (Wmt1, Wmt2 and Wmt3). They differ in their affinity, expression patterns and levels when exposed to metal ions and Wmt2 is the most responsive protein among Wmts especially to Cd. Though earthworm metallothioneins are well studied and documented, the mechanism of gene induction and mechanism of action need more scientific investigation, wMT3 remains the least understood and it is under reported. Also, with the advent of nanotechnology, a handful of studies have evaluated the effect of nanoparticles in the environment using metallothioneins and a few focused-on earthworms an important entity of the soil ecosystem. Nanoparticles ecotoxocological impact are not well elucidated and remains an area that require more research attention. Other specific areas are wMTs induction, mechanism of action and their entire functions in nanoparticle impacted environment. Research is an ongoing process and the grey areas in earthworm metallothioneins research highlighted in this review are area that can be elucidated.

Acknowledgement

We thank the Schlumberger faculty of the future foundation for their support during this work.

References

1. OECD TN. 207: (1984) Earthworm, acute toxicity tests. OECD Guidelines for the Testing of Chemicals 1: 1-9.

2. Dallinger R (1996) Metallothionein research in terrestrial invertebrates: synopsis and perspectives. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 113: 125-133. **Link:** https://bit.ly/35o2iAF

3. Belfroid AC, Sijm DT, Gestel CV (1996) Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environmental Reviews 4: 276-299. **Link:** https://bit.ly/34n3BRm

Citation: Aemere O, Sharma V, Lin J (2020) Metallothioneins in Earthworms: The Journey So Far. Open J Environ Biol 5(1): 014-021.
DOI: https://dx.doi.org/10.17352/ojeb.000016

4. Dickins TE, Dickins BJ (2008) Mother nature's tolerant ways: why non-genetic inheritance has nothing to do with evolution. New Ideas in Psychology 26: 41-54. **Link:** https://bit.ly/3ohSukr

5. Aziz NA, Morgan AJ, Kille P (1999) III. EARTHWORM ECOTOXICOLOGY-Metal resistance in earthworms; genetic adaptation or physiological acclimation. Pedobiologia 43: 594-601.

6. Maboeta MS, Reinecke AJ, Reinecke SA (1999) Effects of low levels of lead on growth and reproduction of the Asian earthworm Perionyx excavatus (Oligochaeta). Ecotoxicology and Environmental Safety 44: 236-240. **Link:** https://bit.ly/35oOjKZ

7. Spurgeon DJ, Hopkin SP (2000) The development of genetically inherited resistance to zinc in laboratory-selected generations of the earthworm Eisenia fetida. Environmental Pollution 109: 193-201. **Link:** https://bit.ly/2Th7KQ6

8. Langdon CJ, Piearce TG, Meharg AA, Semple KT (2003) Interactions between earthworms and arsenic in the soil environment: a review. Environmental Pollution 124: 361-373. **Link:** https://bit.ly/37zOUMr

9. van Gestel CA (2012) Soil ecotoxicology: state of the art and future directions. ZooKeys 275.

10. Novelli EL, Rodriguee NL, Ribas BO (1995) Superoxide radical and toxicity of environmental nickel exposure. Human Exp Toxicol 14: 248–251. **Link:** https://bit.ly/3e21lCh

11. Galay-Burgos M, Spurgeon DJ, Weeks JM, Stürzenbaum SR, Morgan AJ, et al. (2003) Developing a new method for soil pollution monitoring using molecular genetic biomarkers. Biomarkers 8: 229-239. **Link:** https://bit.ly/37uDwBH

12. Stürzenbaum SR (2009) Earthworm and nematode metallothioneins. Metal ions in life sciences 5: 183-197. **Link:** https://rsc.li/3olnjov

13. Sanchez-Hernandez JC, del Pino JN, Domínguez J (2015) Earthworm-induced carboxylesterase activity in soil: Assessing the potential for detoxification and monitoring organophosphorus pesticides. Ecotoxicology and Environmental Safety 122: 303-312. **Link:** https://bit.ly/3okxjOM

14. Mo X, Qiao Y, Sun Z, Sun X, Li Y (2012) Molecular toxicity of earthworms induced by cadmium contaminated soil and biomarkers screening. Journal of Environmental Sciences 24: 1504-1510. **Link:** https://bit.ly/3jpmAPj

15. Bernard F, Brulle F, Douay F, Lemière S, Demuynck S, et al. (2010) Metallic trace element body burdens and gene expression analysis of biomarker candidates in Eisenia fetida, using an “exposure/depuration” experimental scheme with field soils. Ecotoxicol Environ Safety 73: 1034–1045. **Link:** https://bit.ly/3ohRNrl

16. Stürzenbaum SR, Kille P, Morgan AJ (1998) The identification, cloning and characterization of earthworm metallothionein. Febs Letters 431: 437-442. **Link:** https://bit.ly/3olQ446

17. Stürzenbaum SR, Georgiev O, Morgan AJ, Kille P (2004) Cadmium detoxification in earthworms: from genes to cells. Environmental Science & Technology 38: 6283-6289. **Link:** https://bit.ly/3dUgfKv

18. Margoshes M, Vallee BL (1957) A cadmium protein from equine kidney cortex. Journ of the Ame Chem Soc 79: 4813-4814. **Link:** https://bit.ly/3knt2aF

19. Carmona F, Palacios Ò, Gálvez N, Cuesta R, Atrian S, et al. (2013) Ferritin iron uptake and release in the presence of metals and metalloproteins: chemical implications in the brain. Coordination Chemistry Reviews 257: 2752-2764. **Link:** https://bit.ly/3dQeMVv

20. Raudenska M, Gumulec J, Podlaha O, Sztalmachova M, Babula P, et al. (2014) Metallothionein polymorphisms in pathological processes. Metallomics 6: 55-68. **Link:** https://rsc.li/2HtT3Hb

21. Skutkova H, Babula P, Stiborova M, Eckschlager T, Trnkova L, et al. (2012) Structure, polymorphisms and electrochemistry of mammalian metallothioneins–a review. Int J Electrochem Sci 7: 12415-124131. **Link:** https://bit.ly/2FSr4Al

22. Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, et al. (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14: 6044-6066. **Link:** https://bit.ly/37FFnn4

23. Rocha TL, Gomes T, Durigon EG, Bebianno MJ (2016) Subcellular partitioning kinetics, metallothionein response and oxidative damage in the marine mussel Mytilus galloprovincialis exposed to cadmium-based quantum dots. Science of the Total Environment 554: 130-141. **Link:** https://bit.ly/2TjOku8

24. Mackay EA, Overnell J, Dunbar B, Davidson I, Hunziker PE, et al. (1993) Complete amino acid sequences of five dimeric and four monomeric forms of metallothionein from the edible mussel Mytilus edulis. European Journal of Biochemistry 218: 183-194. **Link:** https://bit.ly/3oiJ7B3

25. Dziegiel P, Pula B, Kobierzycki C, Stasiolek M, Podhorska-Okolow M (2016) Metallothionein-3. In *Metallothioneins in Normal and Cancer Cells*. Springer, Cham. **Link:** https://bit.ly/3dOa9eR

26. Sakulsak N (2012) Metallothionein: an overview on its metal homeostatic regulation in mammals. Int J Morphol 30: 1007-1012. **Link:** https://bit.ly/3kp6KFA

27. Wu H, Kong L, Cheng Y, Zhang Z, Wang Y, et al. (2015) Corrigendum to"Metallothionein plays a prominent role in the prevention of diabetic nephropathy by sulforaphane via up-regulation of NF_2 [Free Radic Biol Med 89: 431-42] 97: 621. **Link:** https://bit.ly/3koiYhQ

28. Chidinma NC, Adewale A, Chiaka A (2016) Differential expression of metallothionein-1 and cytochrome p450-2a5 (cyp2a5) in mice in response to lead acetate exposure and industrial effluents in Ibadan, Nigeria. Toxicology and industrial health 32: 1875-1881. **Link:** https://bit.ly/2IQFbXS

29. Ryvolova M, Krizkova S, Adam V, Beklova M, Trnkova L, et al. (2011) Analytical methods for metallothionein detection. Current Analytical Chemistry 7: 243-261. **Link:** https://bit.ly/3oglpoZ

30. Isani G, Carpenè E (2014) Metallothioneins, unconventional proteins from unconventional animals: a long journey from nematodes to mammals. *Biomolecules 4*: 435-457. **Link:** https://bit.ly/3odjcuB

31. Höckner M, Dallinger R, Stürzenbaum SR (2015) Metallothionein gene activation in the earthworm (Lumbricus rubellus). Biochem Biophys Res Commun 460: 537-542. **Link:** https://bit.ly/3koc9Ni

32. Catalán A, Glaser-Schmitt A, Argyridou E, Duchen P, Parsch J (2016) An indel polymorphism in the MtnA 3'untranslated region is associated with gene expression variation and local adaptation in Drosophila melanogaster. PLoS Genetics 12. **Link:** https://bit.ly/31yIoSu

33. Liu X, Wang WX (2016) Time changes in biomarker responses in two species of oyster transplanted into a metal contaminated estuary. Science of the Total Environment 544: 281-290. **Link:** https://bit.ly/34jHjjb

34. Catherine T, Vanessa M, Evangelia S, Valentina C, Andreja R, et al. (2016) Biochemical biomarker responses to pollution in selected sentinel organisms across the Eastern Mediterranean and the Black Sea. Environ Sci Pollut Res Int 23: 1789-1804. **Link:** https://bit.ly/3dQlpqP

35. Baurand PE, Pedrini-Martha V, De Vaufleury A, Niederwanger M, Capelli N, et al. (2015) Differential expression of metallothionein isoforms in terrestrial snail embryos reflects early life stage adaptation to metal stress. PloS one 10. **Link:** https://bit.ly/2IYCpQw

36. Capdevila M, Atrian S (2011) Metallothionein protein evolution: a miniassay. J Biol Inorg Chem 16: 977-989. **Link:** https://bit.ly/3koZWaW

37. Vallee BL (1991) Introduction to metallothionein. Methods in enzymology 205: 3-7.

38. Liu Y, Wu H, Kou L, Liu X, Zhang J, et al. (2014) Two metallothionein genes in Oxya chinensis: molecular characteristics, expression patterns and roles in heavy metal stress. PloS one 9. **Link:** https://bit.ly/31zf4LQ

39. Oliveira VA, Oliveira CS, Mesquita M, Pedroso TF, Costa LM, et al. (2015) Zinc and N-acetylcysteine modify mercury distribution and promote increase in hepatic metallothionein levels. J Trace Elem Med Biol 32: 183-188. **Link:** https://bit.ly/31y9RUp

40. Kowald GR (2012) *Structure and properties of earthworm metallothionein-2* (Doctoral dissertation, University of Warwick). **Link:** https://bit.ly/3mfL1Ax

41. Serén N, Glaberman S, Carretero MA, Chiari Y (2014) Molecular evolution and functional divergence of the metallothionein gene family in vertebrates. Journal of Molecular Evolution 78: 217-233.

42. Ngu TT, Sturzenbaum SR, Stillman MJ (2006) Cadmium binding studies to the earthworm *Lumbricus rubellus* metallothionein by electrospray mass spectrometry and circular dichroism spectroscopy. Biochemical and Biophysical Research Communications 351: 229-233. **Link:** https://bit.ly/34riSAF

43. Hunt CT, Boulanger Y, Fesik SW, Armitage IM (1984) NMR analysis of the structure and metal sequestering properties of metallothioneins. Environmental Health Perspect 54: 135-145. **Link:** https://bit.ly/35uev6U

44. Thit A, Banta GT, Selck H (2015) Bioaccumulation, subcellular distribution and toxicity of sediment-associated copper in the ragworm Nereis diversicolor: The relative importance of aqueous copper, copper oxide nanoparticles and microparticles. Environmental pollution 202: 50-57. **Link:** https://bit.ly/31zgshw

45. Pedrini-Martha V, Niederwanger M, Kopp R, Schnegg R, Dallinger R (2016) Physiological, diurnal and stress-related variability of cadmium-metallothionein gene expression in land snails. PloS One 11. **Link:** https://bit.ly/3meMkQh

46. Kowald GR, Stürzenbaum SR, Blindauer CA (2016) Earthworm Lumbricus rubellus MT-2: metal binding and protein folding of a true cadmium-MT. Int J Mol Sci 17: 65. **Link:** https://bit.ly/31vjV0k

47. Stürzenbaum SR, Winters C, Galay M, Morgan AJ, Kille P (2001) Metal ion trafficking in earthworms Identification of a cadmium-specific metallothionein. J Biol Chem 276: 34013-34018. **Link:** https://bit.ly/3jnFciG

48. Morgan JE, Morgan AJ (1998) The distribution and intracellular compartmentation of metals in the endogeic earthworm Aporrectodea caliginosa sampled from an unpolluted and a metal-contaminated site. Environmental Pollution 99: 167-175. **Link:** https://bit.ly/3kqQnIA

49. Andre J, Charnock J, Stürzenbaum SR, Kille P, Morgan AJ, et al. (2009) Accumulated metal speciation in earthworm populations with multigenerational exposure to metalliferous soils: cell fractionation and high-energy synchrotron analyses. Environ Sci Technol 43: 6822-6829. **Link:** https://bit.ly/31AQLgh

50. Reinecke SA, Prinsloo MW, Reinecke AJ (1999) Resistance of *Eisenia fetida* (Oligochaeta) to cadmium after long-term exposure. Ecotoxicol Environ Saf 42: 75-80. **Link:** https://bit.ly/3jnETEy

51. Manier N, Brulle F, Le Curieux F, Vandenbulcke F, Deram A (2012) Biomarker measurements in *Trifolium repens* and *Eisenia fetida* to assess the toxicity of soil contaminated with landfill leachate: a microcosm study. Ecotoxicol Environ Saf 80: 339-348. **Link:** https://bit.ly/3dR9nhd

52. Homa J, Rorat A, Kruk J, Cocquerelle C, Plytycz B, et al. (2015) Dermal exposure of *Eisenia andrei* earthworms: Effects of heavy metals on metallothionein and phytochelatin synthase gene expressions in coelomocytes. Environ Toxicol Chem 34: 1397-1404. **Link:** https://bit.ly/2TkXPJC

53. Panzarino O, Hyršl P, Dobeš P, Vojtek L, Vernile P, et al. (2016) Rank-based biomarker index to assess cadmium ecotoxicity on the earthworm *Eisenia andrei*. Chemosphere 145: 480-486. **Link:** https://bit.ly/3ohUGZd

54. Ogunlaja A, Sharma V, Lin J (2020) Ex-situ induction of Metallothionein gene in *Libyodrilus violaceous* post cadmium and zinc exposure. Gene Report 20: 100701. **Link:** https://bit.ly/2HuW89y

55. Haap T, Schwarz S, Köhler HR (2016) Metallothionein and Hsp70 trade-off against one another in Daphnia magna cross-tolerance to cadmium and heat stress. Aquatic Toxicol 170: 112-119. **Link:** https://bit.ly/31vEQjJ

56. Sizmur T, Hodson ME (2009) Do earthworms impact metal mobility and availability in soil?–A review. Environmental Pollution 157: 1981-1989. **Link:** https://bit.ly/3dUihu7

57. Günther V, Lindert U, Schaffner W (2012) The taste of heavy metals: gene regulation by MTF-1. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1823: 1416-1425. **Link:** https://bit.ly/2Hxqh8w

58. Heuchel R, Radtke F, Georgiev O, Stark G, Aguet M, et al. (1994) The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. EMBO Journal 13: 2870-2875. **Link:** https://bit.ly/31zl4Ec

59. Höckner M, Stefanon K, Schuler D, Fantur R, De Vaufleury A, et al. (2009) Coping with cadmium exposure in various ways: the two Helicid snails Helix pomatia and Cantareus aspersus share the metal transcription factor-2, but differ in promoter organization and transcription of their Cd-metallothionein genes. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 311: 776-787. **Link:** https://bit.ly/37tfeli

60. Höckner M, Dallinger R, Stürzenbaum SR (2015) Metallothionein gene activation in the earthworm (Lumbricus rubellus). Biochemical and Biophysical Research Communications 460: 537-542. **Link:** https://bit.ly/2IYCP9y

61. Li LZ, Zhou DM, Peijnenburg WJ, Wang P, van Gestel CA, et al. (2010) Uptake pathways and toxicity of Cd and Zn in the earthworm Eisenia fetida. Soil Biology and Biochemistry 42: 1045-1050. **Link:** https://bit.ly/2TfvXXj

62. Mo X, Qiao Y, Sun Z, Sun X, Li Y (2012) Molecular toxicity of earthworms induced by cadmium contaminated soil and biomarkers screening. Journal of Environmental Sciences 24: 1504-1510. **Link:** https://bit.ly/3jpmAPj

63. Colacevich A, Sierra MJ, Borghini F, Millán R, Sanchez-Hernandez JC (2011) Oxidative stress in earthworms short-and long-term exposed to highly Hg-contaminated soils. J Hazard Mater 194: 135-143. **Link:** https://bit.ly/2TiFYmp

64. Calisi A, Lionetto MG, De Lorenzis E, Leomanni A, Schettino T (2016) Metallothionein induction in the coelomic fluid of the earthworm *Lumbricus terrestris* following heavy metal exposure: a short report. Biomed Res Int 2014. **Link:** https://bit.ly/3kqQPXi

65. Stürzenbaum SR, Kille P, Morgan AJ (1998) The identification, cloning and characterization of earthworm metallothionein. Febs Letters 431: 437-442. **Link:** https://bit.ly/2Tjz68A

66. Foster AW, Robinson NJ (2011) Promiscuity and preferences of metallothioneins: the cell rules. BMC Biology 9: 25. **Link:** https://bit.ly/35o6wZ3

67. van Ommen Kloeke AE, Gong P, Ellers J, Roelofs D (2014) Effects of a natural toxin on life history and gene expression of Eisenia andrei. Environmental Toxicology and Chemistry 33: 412-420. **Link:**

68. Wang J, Zhou J, Chen Y, Zhang X, Jin Y, Cui X, et al. (2019) Rapid one-step enzyme immunoassay and lateral flow immunochromatographic assay for colistin in animal feed and food. Journal of Animal Science and Biotechnology 10: 1-0. **Link:** https://bit.ly/35qKJ2X

69. Guo R, Ding X, Zhong X, Gao S, Sun Y (2014) Molecular and ultrastructural insights into the earthworm Eisenia fetida of the assessment of ecotoxicity during colistin exposure. Environ Sci Pollut Res Int 21: 13405-134011. **Link:** https://bit.ly/3jrJ6H7

70. Li Y, Tang H, Hu Y, Wang X, Ai X, et al. (2016) Enrofloxacin at environmentally relevant concentrations enhances uptake and toxicity of cadmium in the earthworm Eisenia fetida in farm soils. Journal of hazardous materials 308: 312-320. **Link:** https://bit.ly/3meQ2JH

71. Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF, Rejeski D, Hull MS, et al. (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein Journal of Nanotechnology 6: 1769-1780. **Link:** https://bit.ly/3jqAxwi

72. Makama S, Piella J, Undas A, Dimmers WJ, Peters R, et al. (2016) Properties of silver nanoparticles influencing their uptake in and toxicity to the earthworm Lumbricus rubellus following exposure in soil. Environmental Pollution 218: 870-878. **Link:** https://bit.ly/2Hqqv1o

73. El-Nour KM, Eftaiha AA, Al-Warthan A, Ammar RA (2010) Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry 3: 135-140.

74. Maurer-Jones MA, Gunsolus IL, Murphy CJ, Haynes CL (2013) Toxicity of engineered nanoparticles in the environment. Anal Chem 85: 3036-3049. **Link:** https://bit.ly/3dOrlkl

75. Gomes SI, Novais SC, Gravato C, Guilhermino L, Scott-Fordsmand JJ, Soares AM, et al. (2012) Effect of Cu-nanoparticles versus one Cu-salt: analysis of stress biomarkers response in Enchytraeus albidus (Oligochaeta). Nanotoxicology 6:134-143. **Link:** https://bit.ly/3ojvB08

76. Saez G, Aye M, De Meo M, Aimé A, Bestel I, et al. (2015) Genotoxic and oxidative responses in coelomocytes of Eisenia fetida and Hediste diversicolor exposed to lipid-coated CdSe/ZnS quantum dots and CdCl2. Environmental Toxicology 30: 918-926. **Link:** https://bit.ly/2Tivofh

77. Unrine JM, Tsyusko OV, Hunyadi SE, Judy JD, Bertsch PM (2010) Effects of particle size on chemical speciation and bioavailability of copper to earthworms (Eisenia fetida) exposed to copper nanoparticles. J Environ Qual 39: 1942-1953. **Link:** https://bit.ly/35jeltu

78. Bigorgne E, Foucaud L, Lapied E, Labille J, Botta C, et al. (2011) Ecotoxicological assessment of TiO2 byproducts on the earthworm Eisenia fetida. Environ Pollut 159: 2698-2705. **Link:** https://bit.ly/2HpTfr2

79. Ribeiro MJ, Maria VL, Scott-Fordsmand JJ, Amorim MJ (2015) Oxidative stress mechanisms caused by Ag nanoparticles (NM300K) are different from those of AgNO3: effects in the soil invertebrate Enchytraeus crypticus. Int J Environ Res Public Health 12: 9589-9602. **Link:** https://bit.ly/2HtPq3T

80. van der Ploeg MJ, Handy RD, Waalewijn-Kool PL, van den Berg JH, Herrera Rivera ZE, et al. (2014) Effects of silver nanoparticles (NM-300K) on Lumbricus rubellus earthworms and particle characterization in relevant test matrices including soil. Environ Toxicol Chem 33: 743-752. **Link:** https://bit.ly/3dSdjhK

81. Choi JS, Park JW (2015) Molecular characterization and toxicological effects of citrate-coated silver nanoparticles in a terrestrial invertebrate, the earthworm (Eisenia fetida). Molecular & Cellular Toxicology 11: 423-431. **Link:** https://bit.ly/2ThdlpA

82. Stern KG (1939) Oppenheimer C. Biological Oxidation.

83. Thompson JA, Cosson RP (1984) An improved electrochemical method for the quantification of metallothioneins in marine organisms. Marine Environmental Research 11: 137-152. **Link:** https://bit.ly/34mSasD

84. Brdička R (1933) A new test for proteins in the presence of cobalt salts in ammonical solutions of ammonium chloride. Collection 5:112.

85. Brdička R, Březina M, Kalous V (1965) Polarography of proteins and its analytical aspects. Talanta 12: 1149-1162. **Link:** https://bit.ly/2Tfz5Cx

86. Mijošek T, Erk M, Filipović MV, Krasnići N, Dragun Z, et al. (2018) Electrochemical Determination of Metallothioneins by the Modified Brdička Procedure as an Analytical Tool in Biomonitoring Studies. Croatica chemica acta 91: 475-480. **Link:** https://bit.ly/2Tii7TX

87. Raspor B, Pavicic J (1996) Electrochemical methods for quantification and characterization of metallothioneins induced in Mytilus galloprovincialis. Fresenius' Journal of Analytical Chemistry 354: 529-534. **Link:** https://bit.ly/35ogM3D

88. Adam V, Petrlova J, Potesil D, Zehnalek J, Sures B, et al. (2005) Study of Metallothionein Modified Electrode Surface Behavior in the Presence of Heavy Metal Ions-Biosensor. Electroanalysis 17: 1649-1657. **Link:** https://bit.ly/2FRnWVe

89. Klaverkamp JF, Wautier K, Baron CL (2000) A modified mercury saturation assay for measuring metallothionein. Aquatic toxicology 50: 13-25.

90. Svendsen C, Hankard PK, Lister LJ, Fishwick SK, Jonker MJ, et al. (2007) Effect of temperature and season on reproduction, neutral red retention and metallothionein responses of earthworms exposed to metals in field soils. Environmental pollution 147: 83-93. **Link:** https://bit.ly/2lUFUHz

91. Bragigand V, Berthet B (2003) Some methodological aspects of metallothionein evaluation. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 134: 55-61. **Link:** https://bit.ly/31BO8Lf

92. Torreggiani A, Tinti A (2010) Raman spectroscopy a promising technique for investigations of metallothioneins. Metallomics 2: 246-260. **Link:** https://bit.ly/3dQ80Pw

93. Ebadi M, Sharma SK, Ghafourifar P, Brown-Borg H, El Refaey H (2005) Peroxynitrite in the pathogenesis of Parkinson's disease and the neuroprotective role of metallothioneins. Methods Enzymol 396: 276-298. **Link:** https://bit.ly/31wWIL6

94. El Hourch M, Dudoit A, Amiard JC (2004) An optimization procedure for determination of metallothionein by square wave cathodic stripping voltammetry: application to marine worms. Analytical and bioanalytical chemistry 378: 776-781. **Link:** https://bit.ly/3dSun6V

95. Petrlova J, Krizkova S, Zitka O, Hubalek J, Prusa R, et al. (2007) Utilizing a chronopotentiometric sensor technique for metallothionein determination in fish tissues and their host parasites. Sensors and Actuators B: Chemical 127: 112-119. **Link:** https://bit.ly/3mrmn07

96. Ndayibagira A, Sunahara GI, Robidoux PY (2007) Rapid isocratic HPLC quantification of metallothionein-like proteins as biomarkers for cadmium exposure in the earthworm Eisenia andrei. Soil Biology and Biochemistry 39: 194-201. **Link:** https://bit.ly/34knHel

97. Alhama J, Romero-Ruiz A, Jebali J, López-Barea J (2011) Total Metallothionein Quantification by Reversed-phase High-Performance Liquid Chromatography coupled to Fluorescence detection after monobromobimane derivatization. Environmental Research Journal 5. **Link:** https://bit.ly/2TiJFsh

98. Geng MJ, Liang SX, Liu W, Jin Y (2014) Quantification of metallothioneins in the earthworm by lomefloxacin–europium (iii) fluorescent probe. Environmental Science: Processes & Impacts 16: 1923-1929. **Link:** https://bit.ly/3kA4viI

99. Qian QM, Wang YS, Zhou B, Xue JH, Li L, et al. (2014) Fluorescence quenching determination of metallothioneins using 8-hydroxyquinoline-5-sulphonic acid–Cd (II) chelate. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 118: 992-998. **Link:** https://bit.ly/34j8ECa

Citation: Aemere O, Sharma V, Lin J (2020) Metallothioneins in Earthworms: The Journey So Far. Open J Environ Biol 5(1): 014-021.
DOI: https://dx.doi.org/10.17352/ojeb.000016