



Research Article

Deciphering the Toxicity of Polycyclic Aromatic Hydrocarbons on *Eisenia Fetida* as Model Soil Fauna

¹Ibrahim Suleiman, ¹Junaidu Sanusi, ²Haruna Yahaya Ismail* and ¹Sanusi Liadi

¹Department of Biology Isa Kaita College of Education Dutsin-Ma, Katsina State, Nigeria

²Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Borno State, Nigeria

*Corresponding author: yismailh@gmail.com, doi.org/10.55639/607.646362

ARTICLE INFO:

ABSTRACT

Keywords:

Eisenia fetida,
Polycyclic
Aromatic Hydrocarbons,
Endo-1,4-Glucanase,
Ligands

Polycyclic aromatic hydrocarbons (PAHs) represent a pervasive class of environmental pollutants with adverse effects on ecosystems and human health. This study was designed to unravel the intricate tapestry of toxicity specifically on *Eisenia fetida*, a keystone species in terrestrial environments using in silico molecular approach. Four PAHs including Benzo [a] pyrene (CID_2336), Anthracene (CID_31423), Pyrene (CID_31423) and Fluoranthene (CID_9154) were selected based on their relative soil abundance and their complexity. Their toxicity due to physicochemical and bioactive properties was assessed using SwissADME. Their inhibitory effect on endo-1,4-glucanase (PDB ID: 3WC3) was also demonstrated using molecular docking analysis. Results on the physicochemical properties of the ligands indicated that anthracene (CID_8418) and benzo [a] pyrene (CID_2336) had the smallest (178.22 g/mol) and largest (252.32 g/mol) molecular weights respectively. The toxicity of PAHs is a function of their molecular weight and sizes. The outcomes also demonstrated the strong mutagenic and tumorigenic potential of each ligand. The remaining PAHs, with the exception of fluoranthene, are similarly extremely irritating when exposed. Furthermore, all of the compounds except for benzo [a] pyrene are not readily reproduced. The ligands are harmful even to human subjects, according to their pharmacokinetics. The results of a molecular docking analysis indicate that CID_2336, which fits into the active cavity of 3WC3 and is stabilized by seven electrostatic contacts, has the lowest free binding energy of -5.15 kcal/mol. The higher free binding energies of the remaining PAHs ranged from -4.63 kcal/mol to -4.31 kcal/mol. With the exception of CID_31423, which had only six electrostatic connections, all of the ligands were stabilized by seven electrostatic interactions. All of the ligands shared identical amino acid residues, with the exception of CID_8418 and CID_9154, which lacked Ser48 and Val122, respectively. According to these results, 3WC3 can be inhibited by all PAHs. Endo-1,4-glucanase is a key enzyme in the digestive and nutrition absorption processes of the earthworm, and its inhibition can have detrimental effects on the organism. The creation of PAHs carrier molecules is necessary to prevent the compounds' negative effects on the worm population during remediation or composting activities.

Corresponding author: Haruna Yahaya Ismail, Email: yismailh@gmail.com

Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Borno State, Nigeria

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are hazardous pollutants that are ubiquitous in the environment. They are formed by the incomplete combustion of organic matter and are found in air, water, soil and sediments. PAHs are known to be toxic to aquatic animals, invertebrates, and soil fauna, including earthworms (Zhang *et al.*, 2021). PAHs are formed by incomplete combustion of organic matter, including fossil fuels, wood and tobacco. They are also found in coal tar, asphalt, and creosote. PAHs can be released into the environment through various sources, including industrial processes, transportation, and natural sources such as forest fires and volcanic eruptions (Agency for Toxic Substances and Disease Registry; ATSDR, 2021). PAHs have a significant impact on the various components of the ecosystem. They can cause harm to aquatic life, including fish and invertebrates, and can also affect the growth and development of plants. PAHs can also lead to soil contamination (Dai *et al.*, 2022), which can have long-term effects on soil quality and ecosystem health (ATSDR, 2021; Zhang *et al.*, 2021). In addition, PAHs are known to be toxic to humans and can cause a range of health effects, including cancer, respiratory problems, developmental and reproductive problems. PAHs can also affect the immune system and are potent immune-suppressants. Exposure to PAHs can occur through inhalation, ingestion and skin contact. The most common sources of exposure include cigarette smoke, air pollution and contaminated food and water (ATSDR, 2021; Zhang *et al.*, 2021).

Remediation of PAHs is a challenging task due to their recalcitrance while in the environment (Song *et al.*, 2023). Various methods have been developed to remediate PAH-contaminated sites, including physical, chemical and biological methods (Ismail *et al.*, 2022). Biological methods, such as bioremediation, involve the use of microorganisms to degrade PAHs into less

toxic compounds. Bioremediation is a promising approach to remediate PAH-contaminated sites, as it is cost-effective and environmentally friendly (ATSDR, 2021; Zhang *et al.*, 2019). One form of bioremediation is vermiremediation which is a process that uses invertebrates particularly worms with a view to eliminating or removing organic and inorganic pollutants most especially in the soil environment (Njoku *et al.*, 2017). An organism of interest is *Eisenia fetida*, and its used in hydrocarbon bioremediation have been fascinating (Deris *et al.*, 2021; Nobili *et al.*, 2022).

Eisenia fetida – a species of earthworm is ubiquitous annelid mostly found in a wide range of soils and may represent over 60% of the total soil biomass (Edwards and Bate, 1992). The worm species have been used as important tool in bioremediation process and remarkable successes were recorded (Chachin *et al.*, 2015; Chachin *et al.*, 2016; Jatwani *et al.*, 2016). This may be due to its ability to accumulate many lipophilic organic pollutants from the surrounding soil environment, by passive absorption through the body wall and also by intestinal uptake during the passage of soil through the gut (Belfroid *et al.*, 1995). A study by Almutairi (2019) showed that *Eisenia fetida* could effectively reduce TPH of about 5000 mg/Kg every 5 weeks. Another study by Liu *et al.* (2017) showed that *Eisenia fetida* could remove PAHs at rate of 90mg/day. Bioremediation using *Eisenia fetida* is a promising approach to remediate PAH-contaminated soil, as it is cost-effective and environmentally friendly. Although success is recorded during remediation process, the population of *Eisenia fetida* tends to be affected negatively at point in time, which might suggest toxicity of pollutants to the organism (Nam *et al.*, 2015; He *et al.*, 2023). Studies have shown that PAHs can have several physiological effects on *Eisenia fetida*, including changes in behaviour, reproduction and mortality (Anyanwu *et al.*, 2017; He *et al.*, 2022). PAHs can affect the behaviour of

Eisenia fetida, leading to changes in burrowing activity, surface activity, and feeding behaviour. A study by Liu *et al.* (2017) showed that exposure to PAHs led to decreased burrowing activity and increased surface activity. Another study by Gomez *et al.* (2011) showed that exposure to PAHs led to decreased feeding activity in *Eisenia fetida*. These changes in behaviour can have significant impacts on the ecological functions of *Eisenia fetida* in soil processes such as organic matter degradation, mineralization and pathogen regulation. PAHs can also affect the reproduction of *Eisenia fetida*, leading to changes in cocoon production and hatching success. Earlier study by Wu *et al.* (2012) showed that exposure to PAHs led to decreased reproductive process in *Eisenia fetida* which might have had significant impacts on the population dynamics of *Eisenia fetida* and its role in soil processes. PAHs can also lead to increased mortality in *Eisenia fetida*. A study by Yang *et al.* (2020) showed that exposure to PAHs led to decreased earthworm biomass and increased mortality. The mechanisms of PAH toxicity on *Eisenia fetida* are complex and involve several pathways, including oxidative stress, DNA damage, and disruption of enzyme activity. A study by Liu *et al.* (2017) showed that exposure to PAHs led to increased oxidative stress in *Eisenia fetida*, as evidenced by increased levels of reactive oxygen species and decreased levels of antioxidant enzymes. Another study by He *et al.* (2022) showed that exposure to PAHs led to DNA damage in *Eisenia fetida*, as evidenced by increased levels of DNA strand breaks. These mechanisms can have significant impacts on the health and survival of *Eisenia fetida*. Despite the toxic effects of PAHs on several cellular functions of *Eisenia fetida*, the organism has been shown to increase the removal of PAHs from soil, indicating its potential use in bioremediation. *Eisenia fetida* is widely used in ecotoxicology studies. It is a

suitable model fauna for studying the effects of PAHs on soil communities because it is easy to culture, has a short life cycle, and is sensitive to environmental stressors. Although there are increasing evidences of PAHs toxicity to *E. fetida*, molecular mechanisms of the toxic effects are still poorly understood. Because *Eisenia fetida* are primarily detritivores, relying heavily on enzymes for digestion of cellulose and survival, the present study explored the *in-silico* interaction of endo-1,4-glucanase (an enzyme that hydrolyzes cellulose by breaking the β -1,4-glycosidic bonds in cellulose molecules) with some of the persistent PAHs. This is with a view to understanding the molecular mechanisms of PAHs toxicity to one of the most essential enzymes that plays crucial role in *Eisenia fetida* physiology and metabolism.

MATERIALS AND METHODS

Protein Structure Retrieval

The crystal structure of endo-1,4-glucanase (Arimori *et al.*, 2013) was retrieved from the Protein Data Bank (PDB) under the accession code 3WC3 (PDB ID: 3WC3). The structure was chosen based on its relevance to the study and quality of resolution. The retrieved protein structure was processed using molecular modeling software to prepare it for molecular docking. Missing parameters including atoms, residues, side chains and missing loops, were verified and attached to their respective sites. In appropriate molecular orientations (chirality) were identified, and disulfide bond and steric clashes checked and corrected. With the exception of water molecules near substrate binding site or non-protein moieties, other water molecules were removed to optimize the protein structure and minimize energy. Chimera, Swiss PDB Viewer and Chiron energy minimization and refinement tool were used (Pettersen *et al.* 2004; Ramachandran *et al.* 2011; Johansson *et al.* 2012). Figure 1 shows the 3D structure of the 3WC3 protein as retrieved from the PDB.

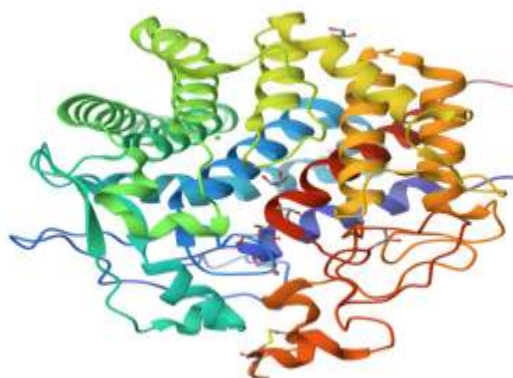


Figure 1: The 3D structure of the enzyme Endo-1,4-glucanase (3WC3)

Ligand Preparation

Polycyclic Aromatic Hydrocarbons (PAHs) used as ligands in this study were obtained from PubChem and the 3D structures of PAHs were generated and energy-minimized using Schrödinger Suite (Bhachoo and Beuming, 2017). The ligands were converted into the

PDB format. The selected ligands were: Benzo [a] pyrene (CID_2336), anthracene (CID_31423), pyrene (CID_31423) and Fluoranthene (CID_9154) as shown in Table 1 below and Figure 2 shows their structural 3D structural representation.

Table 1: Polycyclic aromatic hydrocarbons retrieved from PubChem

S/N	PubChem ID	Compound Name	Formula	Number of Rings	Molecular Weight
1.	CID_8418	Anthracene	C ₁₄ H ₁₀	3	178.233
2.	CID_31423	Pyrene	C ₁₆ H ₁₀	4	202.255
3.	CID_9154	Fluoranthene	C ₁₆ H ₁₀	4	202.255
4.	CID_2336	Benzo[a]pyrene	C ₂₀ H ₁₂	5	252.315



Figure 2: The 3D structures of selected PAHs to be used as ligands for the inhibition of 3WC3 a). anthracene, b). pyrene c). fluoranthene d). benzo[a]pyrene

Physicochemical Analysis

All the selected PAHs were screened for physicochemical properties to determine their activities using Lipinski rule of seven (Molecular weight, logarithms of partial coefficient, hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA)) using SwissADME as described by Isa *et al.* (2022).

Toxicity Study

The ability of the ligands to induce toxicity was evaluated using SwissADME (Daina *et al.* 2017). In addition to that, other exposure-related sequelae such as cytochrome P450 (CYP450) inhibition, mutagenicity, tumorigenicity, Ames toxicity, and reproduction were also determined.

Molecular Docking Analysis

Using AutoDock4.2, the molecular docking analysis was carried out to determine the binding affinity of the protein-ligand complexes. The binding conformation would help to show the 3WC3 and chosen ligands' binding energies. Docking conditions and set up were as described elsewhere by Isa *et al.* (2022). There were a total of 10 runs, with a population size of 150, a maximum generation of 27,000, and a maximum evaluation of 2,500,000. The sum of the van der Waals energy (ΔG_{vdw}), electrostatic energy (ΔG_{elect}), hydrogen bond and desolvation energy (ΔG_{hbond}), final total internal energy ($\Delta G_{\text{conform}}$), torsional free energy (ΔG_{tor}), and unbound system energy (ΔG_{solv}) was used to calculate the free binding energy (ΔG_{bind}).

RESULTS AND DISCUSSION

Investigation into the properties, activity and interaction of four important PAHs was carried out with a view to elucidating the molecular basis of their toxicity. Results on the physicochemical properties of the ligands indicated that anthracene (CID_8418) and benzo [a] pyrene (CID_2336) had the smallest (178.22 g/mol) and largest (252.32 g/mol) molecular weights respectively. On the other hand, each of fluoranthene (CID_9154) and pyrene (CID_32423) had 202.26 g/mol molecular weight as shown in Table 2. The size and complexity of PAHs increase along with their molecular weight. The bigger PAHs like benzo[a]pyrene (CID_2336) have numerous fused rings, smaller PAHs like naphthalene (CID_931) only have two fused benzene rings. The physicochemical characteristics of PAHs are a function of their size and molecular weights (Achten and Andersson, 2005). Typically, low vapour pressure, low water solubility, and high melting and boiling points characterize the majority of PAHs. Since their resistance to oxidation and reduction increases with molecular weight, the water solubility and vapor pressure tend to decrease as well (Eldos *et al.*, 2022). This renders most of the PAHs

hydrophobic; soluble only in organic solvents. Individual PAHs' physicochemical characteristics influence their fate, behaviour, and transit in different environments and tissues. Based on their chemical characteristics and environmental factors (such as temperature), PAHs will migrate between environmental matrices in very different ways. As a result, their concentration varies from one location to the next (Chen *et al.*, 2020).

Similarly, as molecular weight is proportional to molecular size, larger ligands can have greater binding affinity if they can establish multiple interactions with a protein during ligand-protein complex formation. Smaller ligands on the other hand, may have lower binding affinity due to fewer interaction opportunities (Carlson *et al.*, 2008). In addition, larger ligands may have a higher probability of binding to multiple biological targets due to their size and increased potential for interactions, even though it may lead to off-target effects and increased toxicity if those unintended interactions result in adverse biological responses (Ma *et al.*, 2009).

Results in Table 2 also indicated that the selected ligands are neither hydrogen bond acceptors nor hydrogen bond donors and none of the ligands has rotatable bonds also. This is a good indication of the ligands' stability and rigidity respectively which may enable them to form stronger and more specific bonds within the protein's active site. Although hydrogen bonds play a crucial role in ligand-protein complex formation, ligands that cannot form hydrogen bonds may rely on other types of interactions, such as hydrophobic interactions or van der Waals forces, to bind to their target proteins; which can affect the specificity and strength of the ligand-protein interaction (Chen and Kurgan, 2009). The conformation of ligands without rotatable bonds is stable and inflexible. Ligands in such complexes are unable to modify their shape to fit various binding sites or to a target molecule's structure. As a result, the ligand can only successfully bind to particular target sites that exactly fit its

conformation. This is desirable especially if a stiff ligand fits properly into the binding site, it may have powerful binding interactions with its target. A stable ligand-receptor complex with potent non-covalent interactions like van der Waals forces can be produced by the lack of conformational flexibility (Tuffery and Derreumaux, 2012). Studies have shown that high specificity in binding interactions may be facilitated by the stiffness of the ligand. The ligand may show extremely high affinity and selectivity for the target site if its form is a perfect match (Lawson *et al.*, 2018).

The molar refractivity ranged between 61.45 m³/mol in anthracene and 87.65 m³/mol in benzo [a] pyrene. Although fluoranthene (68.38 m³/mol) and pyrene (70.15 m³/mol) have similar molecular weight, their molar

refractivity differed. Although molar refractivity (MR) is not a direct factor that affects ligand-protein interactions, its relationship to the polarizability and size of a ligand is a strong point of interest. As the ligands have high MR, their solubility is extremely low if at all they can be soluble in aqueous substances. Solubility can impact how well a ligand is distributed in a biological system and its bioavailability, which can indirectly affect its interaction with a protein target (Papanephytous *et al.*, 2012). With the exception of fluoranthene (-2.1841), the remaining PAHs had -2.3575 as the value for their drug likeness. This indicates that all the ligands are capable of penetrating membrane and readily available within biological system.

Table 2: Physicochemical properties of the selected PAHs ligands

PubChem ID	Molecular weight (≤700)	Number of HBA (≤7)	Number of HBD (≤7)	Mol LogP (≤7)	Drug likeness	Molar refractivity	Rotatable bonds
CID_2336	252.315	0	0	5.884	-2.3575	87.65	0.00
CID_8418	178.233	0	0	4.048	-2.3575	61.45	0.00
CID_9154	202.255	0	0	4.513	-2.1841	68.38	0.00
CID_31423	202.255	0	0	4.689	-2.3575	70.15	0.00

Table 3: Pharmacokinetic analysis of the selected PAHs ligands

S/No.	PAHs	CYP2D6 inhibitor	Mutagens	Tumorigens	Reproducibility	Irritant
1	CID_2336	Non Inhibitor	High	High	High	High
2	CID_8418	Non Inhibitor	High	High	None	High
3	CID_9154	Non Inhibitor	High	High	None	None
4	CID_31423	Non Inhibitor	High	High	None	High

Results in Table 3 show that all the ligands are highly mutagenic and tumorigenic. With the exception of fluoranthene, the remaining PAHs are highly irritating upon exposure also. More so, the compounds are none reproducible except benzo [a] pyrene, which is highly reproducible. The pharmacokinetics of the ligands indicates that they are toxic even in human subject. With fragility and lack of complex tissue organization in *Eisenia fetida* like that of humans, the toxic effects of the ligands on the invertebrate's tissues may likely

be more severe. In a recent study on acute and chronic toxicity of grapheme oxide to *Eisenia fetida*, serious damages in its skin and intestine with increasing concentration was reported (Duo *et al.*, 2022). In addition, the growth and reproduction of earthworms were significantly inhibited after the exposure. Conversely, results on the ability of the PAHs to inhibit cytochrome P450 were all negative. This however, did not preclude such possibility because the CYP2D6 referred here is a cytochrome of humans. It has been

demonstrated that *Eisenia fetida* also has a specific cytochrome P450 which is been expressed upon exposure to stress (Li *et al.*, 2018). Its role in biodegradation of perfluorooctane sulfonamide has recently been reported (Zhao *et al.*, 2020). However, Liu *et*

al. (2021) showed that the CYP450 of *Eisenia fetida* could be readily disrupted by compounds such as enantiomers of hexaconazole (*S*-(+)-HEX) – an antifungal agent.

Table 4: Docking scores and residues involved in bond formation

S/No.	Compound ID	Docking score (Kcal/mol)	Residues involve in H-bonds	Distance (Å)	Electrostatic Interaction
1	CID_2336	-5.15	0	0	Ser48, Leu47, Ala120, Ala121, Leu123, Ile238, Ser242
2	CID_8418	-4.31	0	0	Val122, Leu123, Ile238, Ser242, Leu47, Ala120, Ala121
3	CID_9154	-4.63	0	0	Leu47, Ser242, Ile238, Leu123, Val122, Ala121, Ala120
4	CID_31423	-4.32	0	0	Ser242, Leu123, Val122, Ala121, Leu47, Ser48

Molecular docking analysis shows that CID_2336 had the least free binding energy of -5.15 kcal/mol and fits to the active cavity of 3WC3 and stabilized by 7 electrostatic interactions at Ser48, Leu47, Ala120, Ala121, Leu123, Ile238 and Ser242. The electrostatic interactions are van der Waals forces, Pi-Sigma, Pi-Lone Pair and Pi-Alkyl interactions. The remaining PAHs had higher free binding energies ranging between -4.63 kcal/mol and -4.31 kcal/mol. Each of the ligands however, were stabilized by 7 electrostatic interactions with the exception of CID_31423 which had only 6. In addition, all the ligands shared similar amino acid residues except Ser48

which was absent in CID_8418 and CID_9154 which had Val122 instead. In CID_31423, the electrostatic interactions were at Leu47, Ser48, Ala121, Val122, Leu123 and Ser242 as shown in Table 4. These findings indicated that all the PAHs are capable of inhibiting 3WC3. Inhibiting endo-1,4-glucanase in *Eisenia fetida* can have serious negative consequences on the organism, as this enzyme plays a crucial role in the earthworm's digestion and nutrient absorption processes. This severely affects the earthworm's growth, reproduction, and overall health. Figure 3 and Figure 4 show the 3D and 2D interaction of the ligands to the 3WC3 enzyme respectively.

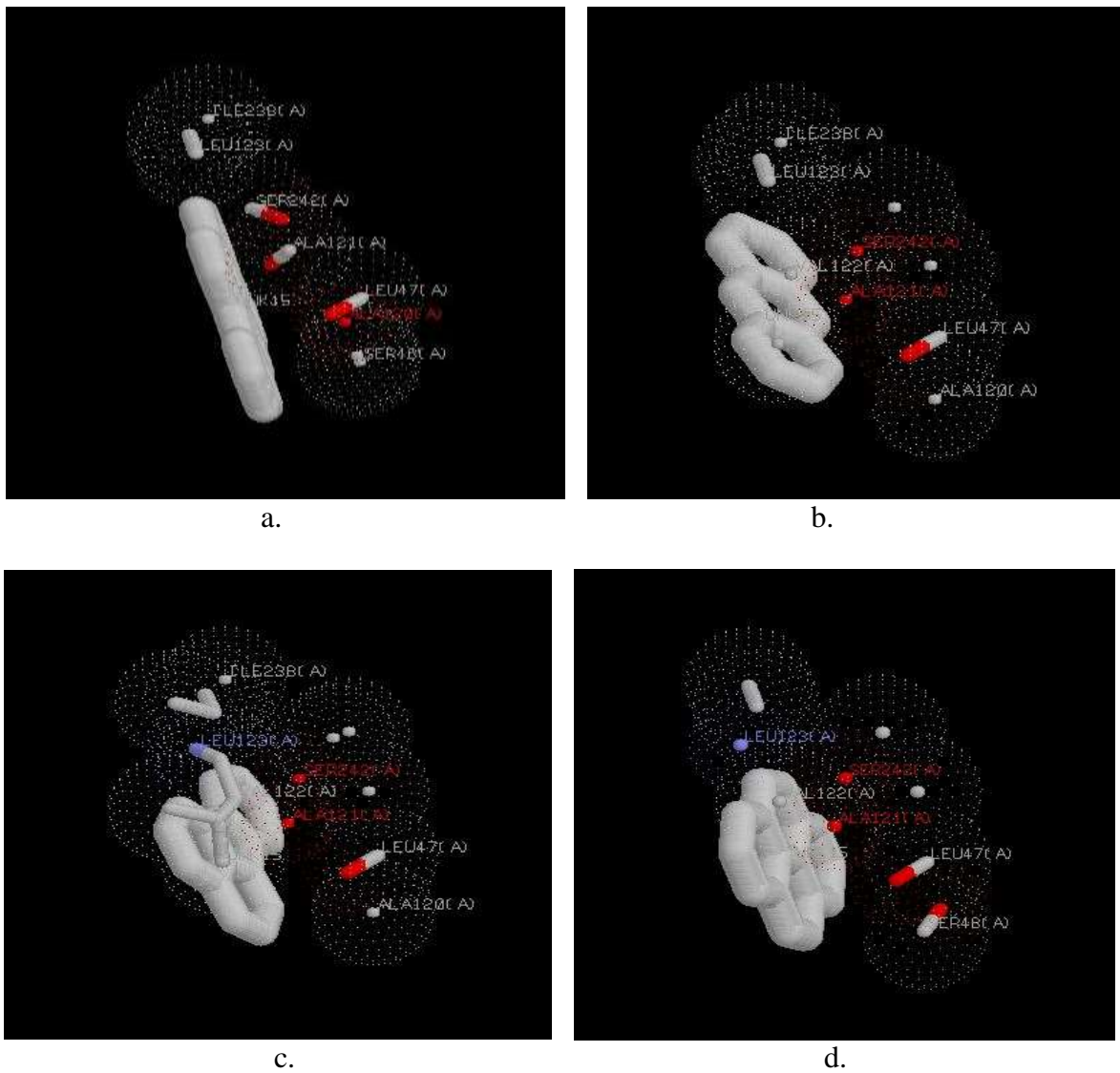
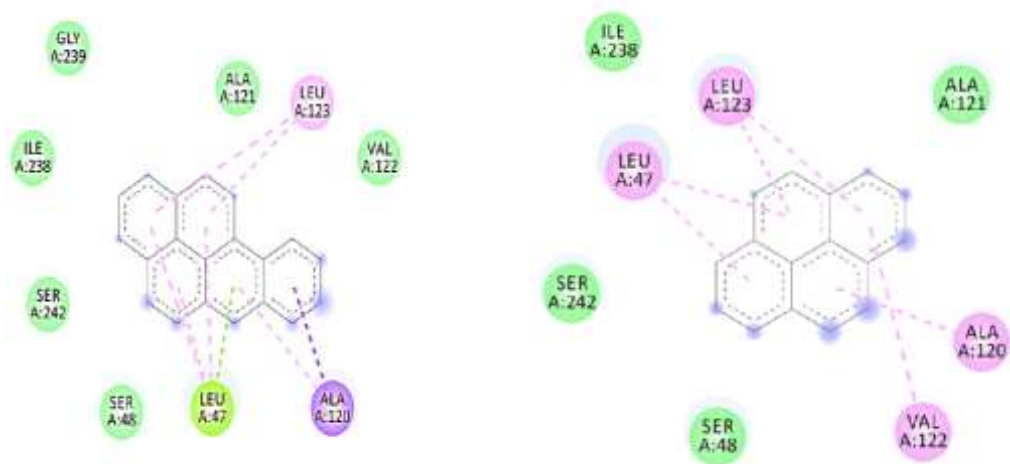


Figure 3: A 3D representation of residues involved in bond formation and electrostatic interactions of the protein ligand complexes (a) benzo [a] pyrene (b) anthracene (c) fluoranthene (d) pyrene



b.

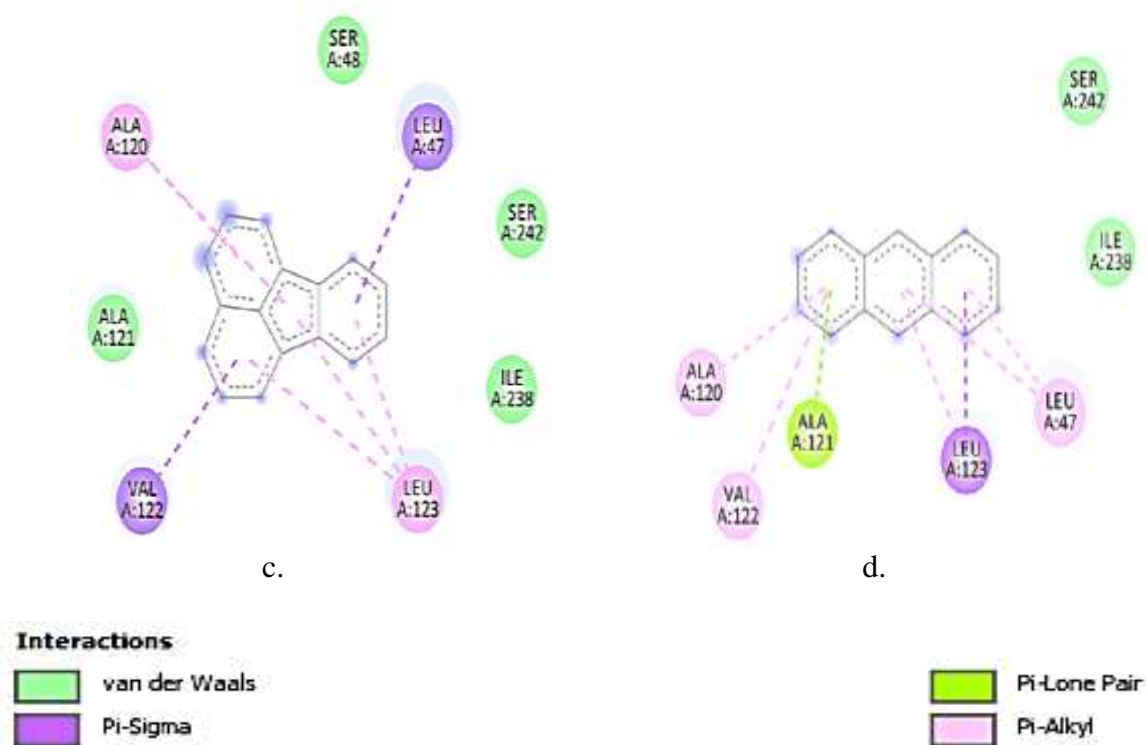


Figure 4: A 2D representation of the electrostatic interactions between the ligands and 3WC3 (a). Benzo [a] pyrene (b). Pyrene (c). Fluoranthene (d). Anthracene

CONCLUSION

In deciphering the toxicity of Polycyclic Aromatic Hydrocarbons on *Eisenia fetida*, the results of the present study indicated that all the selected PAHs are capable of inducing toxicity to the organism. The findings highlighted that the toxicity could be mediated through multifaceted dimensions ranging from the physicochemical properties of the compounds, their biological activity and ability to inhibit the activity of one of the most-significant enzyme of *Eisenia fetida*. The results indicated that the compounds were not only toxicogenic, but they were also highly mutagenic and tumorigenic. Benzo [a] pyrene could be more toxic considering its molecular weight, drug-likeness and lower free binding energy during protein-ligand complex formation. The findings underscored the intricate mechanisms underlying the toxicity of PAHs to *Eisenia fetida*. As incorporation of worms during vermiremediation is necessary,

developing of PAHs carrier molecules is essential in order to avoid the adverse effect of the compounds on the population of worms during remediation exercise.

FUNDING

The research was funded through the IBR grants provided by Tertiary Education Trust Fund (Tetfund), Nigeria through the Isa Kaita College of Education, 2023.

REFERENCES

- Achten, C., & Andersson, J. T. (2015). Overview of polycyclic aromatic compounds (PAC). *Polycyclic Aromatic Compounds*, 35(2-4), 177-186.
- Agency for Toxic Substances and Disease Registry. (2021). Polycyclic Aromatic Hydrocarbons (PAHs). Retrieved from <https://www.atsdr.cdc.gov/toxfaqs/faqsp?id=121&tid=25>
- Almutairi, M. Vermiremediation strategy for remediation of Kuwaiti oil

- contaminated soil. *Springer Nature Applied Science* 1, 1312 (2019).
- Anyanwu, I. N., Clifford, O. I., & Semple, K. T. (2017). Effects of single, binary and quinary mixtures of phenanthrene and its N-PAHs on *Eisenia fetida* in soil. *Water, Air, & Soil Pollution*, 228, 1-10.
- Arimori, T., Ito, A., Nakazawa, M., Ueda, M., & Tamada, T. (2013). Crystal structure of endo-1,4- β -glucanase from *Eisenia fetida*. *Journal of Synchrotron Radiation*, 20, 884–889.
- Bhachoo, J., & Beuming, T. (2017). Investigating protein–peptide interactions using the Schrödinger computational suite. *Modeling peptide-protein interactions: methods and protocols*, 235-254.
- Carlson, H. A., Smith, R. D., Khazanov, N. A., Kirchhoff, P. D., Dunbar, J. B., & Benson, M. L. (2008). Differences between High- and Low-Affinity Complexes of Enzymes and Nonenzymes. *Journal of Medicinal Chemistry*, 51(20), 6432–6441.
- Chachina S.B., Voronkova N. A. and Baklanova O.N. (2016). Biological remediation of the petroleum and diesel contaminated soil with earthworms *Eisenia fetida*. *Procedia Engineering*, 152, 122 – 133.
- Chachina, S. B., Voronkova N. A. and Baklanova O.N. (2015). Biological remediation of the engine lubricant oil-contaminated soil with three kinds of earthworms, *Eisenia fetida*, *Eisenia andrei*, *Dendrobena veneta*, and a mixture of microorganisms. *Procedia Engineering*, 113, 113 – 123.
- Chen, C. F., Ju, Y. R., Su, Y. C., Lim, Y. C., Kao, C. M., Chen, C. W., & Dong, C. D. (2020). Distribution, sources, and behavior of PAHs in estuarine water systems exemplified by Salt River, Taiwan. *Marine Pollution Bulletin*, 154, 111029.
- Chen, K., & Kurgan, L. (2009). Investigation of atomic level patterns in protein-small ligand interactions. *PLoS one*, 4(2), e4473.
- Dai, C., Han, Y., Duan, Y., Lai, X., Fu, R., Liu, S., & Zhou, L. (2022). Review on the contamination and remediation of polycyclic aromatic hydrocarbons (PAHs) in coastal soil and sediments. *Environmental Research*, 205, 112423.
- Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7:42717.
- Deris, M., & Miroliaei, M. R. (2021). Toxic hydrocarbon removal of contaminated soil using *Eisenia fetida* with response surface methodology. *Advances in Environmental Technology*, 7(3), 137-151.
- Duo, L., Wang, Y., & Zhao, S. (2022). Individual and histopathological responses of the earthworm (*Eisenia fetida*) to graphene oxide exposure. *Ecotoxicology and Environmental Safety*, 229, 113076.
- Edwards, C. A., & Bater, J. E. (1992). The use of earthworms in environmental management. *Soil Biology and Biochemistry*, 24(12), 1683-1689.
- Eldos, H. I., Zouari, N., Saeed, S., & Al-Ghouti, M. A. (2022). Recent advances in the treatment of PAHs in the environment: Application of nanomaterial-based technologies. *Arabian Journal of Chemistry*, 15(7), 103918.
- Gomez-Eyles, J. L., Sizmur, T., Collins, C. D., & Hodson, M. E. (2011). Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. *Environmental pollution*, 159(2), 616-622.
- He, F., Hu, S., Liu, R., Li, X., Guo, S., Wang, H., ... & Wang, T. (2023). Decoding the biological toxicity of phenanthrene on intestinal cells of *Eisenia fetida*: Effects, toxicity pathways and corresponding mechanisms. *Science of The Total Environment*, 166903.
- He, F., Li, X., Huo, C., Chu, S., Cui, Z., Li, Y., & Liu, R. (2022b). Evaluation of fluorene-caused ecotoxicological responses and the mechanism underlying its toxicity in *Eisenia fetida*: Multi-level analysis of

- biological organization. *Journal of Hazardous Materials*, 437, 129342.
- He, F., Yu, H., Shi, H., Li, X., Chu, S., Huo, C., & Liu, R. (2022a). Behavioral, histopathological, genetic, and organism-wide responses to phenanthrene-induced oxidative stress in *Eisenia fetida* earthworms in natural soil microcosms. *Environmental Science and Pollution Research*, 29(26), 40012-40028.
- Isa, M. A., Mustapha, A., Qazi, S., Raza, K., Allamin, I. A., Ibrahim, M. M., & Mohammed, M. M. (2020). *In silico* molecular docking and molecular dynamic simulation of potential inhibitors of 3C-like main proteinase (3CLpro) from severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) using selected African medicinal plants. *Advances in Traditional Medicine*. 22, pages107–123.
- Ismail, H. Y., Grema, M. N., Isa, M. A., Allamin, I. A., Bukar, U. A. (2022). Petroleum Hydrocarbon Contamination: Its Effects and Treatment Approaches – A Mini Review. *Arid Zone Journal of Basic and Applied Research*, 1 (6), 81-9.
- Jatwani, C., Gupta, R. K., Rai. R. and Bansal, N. (2016). Effects of Hg/Co Toxicity in Soil on Biomolecules of Earthworm, *Eisenia fetida*. *Procedia Environmental Sciences*, 35:450 – 455.
- Johansson MU, Zoete V, Michielin O, Guex N (2012) Defining and searching for structural motifs using DeepView/Swiss-PdbViewer. *BMC Bioinform* 13(1):173.
- Lawson A. D. G., Maccoss M., and Heer J. P. (2018). Importance of rigidity in designing small molecule drugs to tackle protein-protein interactions (PPIs) through stabilization of desired conformers. *Journal of Medicinal Chemistry* 61, 4283–4289..
- Li, Y., Zhao, C., Lu, X., Ai, X., & Qiu, J. (2018). Identification of a cytochrome P450 gene in the earthworm *Eisenia fetida* and its mRNA expression under enrofloxacin stress. *Ecotoxicology and Environmental Safety*, 150, 70-75.
- Liu, T., Fang, K., Liu, Y., Zhang, X., Han, L., & Wang, X. (2021). Enantioselective residues and toxicity effects of the chiral triazole fungicide hexaconazole in earthworms (*Eisenia fetida*). *Environmental Pollution*, 270, 116269.
- Liu, T., Wang, X., You, X., Chen, D., Li, Y., & Wang, F. (2017). Oxidative stress and gene expression of earthworm (*Eisenia fetida*) to clothianidin. *Ecotoxicology and Environmental Safety*, 142, 489-496.
- Ma, B., Shatsky, M., Wolfson, H. J., & Nussinov, R. (2009). Multiple diverse ligands binding at a single protein site: A matter of pre-existing populations. *Protein Science*, 11(2), 184–197.
- Nam, T. H., Jeon, H. J., Mo, H. H., Cho, K., Ok, Y. S., & Lee, S. E. (2015). Determination of biomarkers for polycyclic aromatic hydrocarbons (PAHs) toxicity to earthworm (*Eisenia fetida*). *Environmental Geochemistry and Health*, 37, 943-951.
- Njoku, K. L., Nomba, E. U., & Olatunde, A. M. (2017). Vermiremediation of crude oil contaminated soil using *Eudrillus euginae* and *Lumbricus terrestris*.
- Nobili, S., Masin, C. E., Zalazar, C. S., & Lescano, M. R. (2022). Bioremediation of hydrocarbon contaminated soil using local organic materials and earthworms. *Environmental Pollution*, 314, 120169.
- Papaneophytou, C. P., Mettou, A. K., Rinotas, V., Douni, E., & Kontopidis, G. A. (2012). Solvent selection for insoluble ligands, a challenge for biological assay development: A TNF- α /SPD304 Study. *ACS Medicinal Chemistry Letters*, 4(1), 137–141.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., & Ferrin, TE (2004) UCSF Chimera-a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25(13),1605–1612.
- Ramachandran S, Kota P, Ding F, Dokholyan NV (2011) Automated minimization of steric clashes in protein structures.

- Protein Structure, Function and Bioinformatics*, 9(1):261–270.
- Song, Q., Li, X., Zhao, Z., Li, D., Li, Y., & Hou, N. (2023). Insights into ecotoxicity effects of PAHs and ecosystem responses of remediation strategies under cold stress: From PAHs degradation to ecological restoration regulated by signal molecular. *Chemical Engineering Journal*, 146042.
- Tuffery, P. and Derreumaux, P. (2012). Flexibility and binding affinity in protein–ligand, protein–protein and multi-component protein interactions: limitations of current computational approaches, *Journal of the Royal Society of Interface*.920–933.
- Wu, S., Zhang, H., Zhao, S., Wang, J., Li, H., & Chen, J. (2012). Biomarker responses of earthworms (*Eisenia fetida*) exposed to phenanthrene and pyrene both singly and combined in microcosms. *Chemosphere*, 87(4), 285-293.
- Yang, W., Hadibarata, T., Mahmoud, A. H., & Yuniarto, A. (2020). Biotransformation of pyrene in soil in the presence of earthworm *Eisenia fetida*. *Environmental Technology & Innovation*, 18, 100701.
- Zhang, Q., Saleem, M., & Wang, C. (2019). Effects of biochar on the earthworm (*Eisenia foetida*) in soil contaminated with and/or without pesticide mesotrione. *Science of The Total Environment*, 671, 52-58.
- Zhang, Y., Li, Y., Li, X., & Zhang, Y. (2021). Effects of polycyclic aromatic hydrocarbons on soil microbial communities: A review. *Environmental Pollution*, 268(Pt A), 115766.
- Zhao, S., Wang, B., Zhong, Z., Liu, T., Liang, T., & Zhan, J. (2020). Contributions of enzymes and gut microbes to biotransformation of perfluorooctane sulfonamide in earthworms (*Eisenia fetida*). *Chemosphere*, 238, 124619.