International Journal of Advances in Engineering and Management (IJAEM)
Volume 3, Issue 7 July 2021, pp: 317-325 www.ijaem.net ISSN: 2395-5252

Toxicity of Ternary Mixtures of Phenol, Zinc and Cadmium to Cryptococcus Sp and Saprochetae Sp

Nlemolisa, O.R, Kemka,U.N., Nwokorie, R.C. and Ndu, F.C.
Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria

Submitted: 15-06-2021 Revised: 27-06-2021 Accepted: 30-06-2021

ABSTRACT
With the advent of modern technology and industrialization, environmental pollution of the natural resources by chemicals has become a serious concern. Most of these pollutants are released into the environment as a mixture of organic and inorganic pollutants Organic pollutants released to the environment as a result of these activities include phenol, polycyclic aromatic hydrocarbons, and heavy metals. The present study was carried out to determine the toxicity of the ternary mixtures of zinc, cadmium and phenol to Saprochaete sp. and Cryptococcus sp. The toxicity of phenol, zinc and cadmium were determined based on the inhibition of dehydrogenase activity of Saprochaete sp. and Cryptococcus sp. Fixed ratio design, uniform design concentration ratios (UDCR) and equieffect concentration ratios (EECR) mixtures were designed to evaluate the combined toxicities of these heavy metal ions and phenol. The toxicity thresholds (IC_{50}) were estimated using 3-parameter logistic dose-response model. The ternary mixtures of zinc, phenol and cadmium showed progressive inhibitory effects on the dehydrogenase (enzyme) activity of Cryptococcus sp and Saprochaete sp as the concentration of the mixtures increases. The combined effect of the chemicals on the enzyme activities of Saprochaete sp. and Cryptococcus sp. were determined using toxic index (TI) model. The toxic interaction of all the mixtures ratios showed synergistic interaction for both Saprochaete sp and Cryptococcus sp. The amount of these toxicants present in the environment, affect its toxicity to microorganisms.

Keywords: Heavy metals, Phenol, Ternary mixture, Dehydrogenase activity.

I. INTRODUCTION

Rapid industrialization and urbanization have caused contamination of the environment by phenol and heavy metals, and their rates of mobilization and transport in the environment have greatly accelerated since 1940s [1]. One of the major challenges in the modern society is environmental pollution. Environmental pollution by heavy metals and phenol is a threat to the environment and is of serious concern [2]. Heavy metals are of economic significance and the most important pollutants in the environment by industries. Environmental pollution by heavy metals is serious threat to living organisms in the ecosystem [3] [4] [5]. The bioaccumulation and nonbiodegradability nature of metal toxicity is of great environmental concern [6]. Several inorganic metals like magnesium (Mg), nickel (Ni), chromium (Cr^{3+}), copper (Cu), calcium (Ca), manganese (Mn), and sodium (Na) as well as zinc (Zn) are vital elements needed in small quantity for metabolic and redox functions. Heavy metals such as aluminium (Al), lead (Pb), cadmium (Cd), gold (Au), mercury (Hg), and silver (Ag) do not have any biological role and are toxic to living organisms [4] [7] [8].

Soil is polluted with heavy metals and Cadmium is lethal metal for plant growth and metabolism [9]. Cadmium as a pollutant enters the environment either through geogenic or anthropogenic sources [10]. Geogenic sources mainly comprise of ores of Zinc and Lead, weathering of rocks, volcanic eruptions, and forest fires [11] [12].

Heavy metal toxicity is the ability of a metal to cause detrimental effects on microorganisms, and it depends on the bioavailability of heavy metal and the absorbed dose [13]. Heavy metal toxicity involves several mechanisms, which includes; breaking fatal enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein [13] [14].

Phenol is a common industrial substance involve in the production of chemicals such as xylenols, oils, plastics [15], aspirin [16], antiseptics [17], pharmaceutical, oil refining [18], explosive, dyes [19], reagent in chemical analysis and leather and wood preservatives [20] [21]. Human activities

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are the major contributor to phenol pollution in the environment; especially in soil because its mobility was limited than in an aqueous environment due to high adsorption onto solid surfaces [22] [23]. Soils areas within sawmills are usually highly contaminated with phenolic compounds because they are commonly used to produce wood preservatives [24]. Phenol is considered as the main concern pollutant because of the harmful and toxic effect, and can be accumulated in living organism. Microorganism is important in aquatic ecosystem as the producer that involved in maintaining web chain and nutrient cycle in earth. In the past decades, many researchers are aware of the possibilities of phenol tolerant organism needed in the future especially to obtain non-polluted water sources, thus many various types of microorganisms isolated example: yeast, fungus, bacteria were found that can help in the degradation of phenol in environment [18] [25] [26]. According to Nwanyanwu and Abu, [27], phenol can affects the metabolic process of microorganisms and other organisms which can leads to death if they are unable to acclimatize it. In this study, we investigated the toxicity of ternary mixtures of phenol, zinc and cadmium to Cryptococcus sp and Saprochetae sp.

II. MATERIALS AND METHOD

2.1 Reagents

Salts of heavy metals used in this study are ZnSO4 .7H2O and CdSO4, which were used as source of the heavy metal ions, Zn and Cd ions respectively. Phenol used was obtained from Sigma-chemical Co. USA. The 2, 3, 5-triphenyltetrazolium chloride (TTC) was purchased from Sigma (Germany). Stock solutions of 10 mM of the individual metal ion were prepared in deionized distilled water. The stock solution of 100 mM of phenol was prepared in deionized distilled water.

2.2 Test Organism

The test organisms, Cryptococcus sp and Saprochetae sp. were isolated from hydrocarbon impacted soil [28].

2.3 Preparation of Inoculum

Cryptococcus sp. and Saprochetae sp. cells were cultured in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2℃) for 48 h. The cells were harvested from the culture by centrifugation (ALPIN MEDICAL ENGLAND90 (1)) at 6000 rpm for 10 mins. The harvested cell pellet was washed twice in sterile distilled water by repeated centrifugation (x2) to ensure that there are no leftover of nutrient broth. The washed cells were suspended in sterile deionized distilled water and the optical density (OD) adjusted to 0.2 at wavelength of 600 nm using spectrophotometer (VIS spectrophotometer 721D, Life Assistance Scientific INST. CO) [29]. The cell suspensions were used as inoculum in the toxicity assay.

2.3 Design of Ternary Mixture Ratios

The toxicity of the ternary mixture of zinc, cadmium and phenol were determined using fixed ratio design, uniform design concentration ratios (UDCR) and equieffect concentration ratios (EECR). In each case, the mixture ratio is kept constant; the total concentrations of the mixture were varied in order to obtain a complete dose-response relationship of the mixture. Ternary mixture ratio of zinc, phenol and cadmium were, 12.9% zinc +86.2% phenol +0.9% cadmium, 27.2% zinc + 71.1% phenol +1.7% cadmium, 50% zinc + 45% phenol +5% cadmium and 28% zinc + 70% phenol + 2% cadmium. The equi effect concentration studied was equi-effect concentration 50% (EE50) that is the concentration that inhibited 50% of the yeast enzyme activity. The mixtures were prepared as 100 mM for phenol and 10 mM for Zn and Cd stock solutions by mixing requisite volumes of the individual solution to give a specific concentration ratio. Each mixture was treated as single solution.

2.4 Toxicity assay

The assay for dehydrogenase activity was modified from Nlemolisa et al. (2020). 2, 3, 5-triphenyltetrazolium chloride (TTC) was used as an artificial electron acceptor which was reduced to the red-coloured triphenylformazan (TPF). The reaction mixture consisted of 2-ml final volume of nutrient broth (pH7) and TTC supplemented with varying concentrations the mixtures. Into each tube, 0.5 ml of x4-strength (0.2% w/v) of nutrient broth, required volume of the composite mixture and sterile deionized distilled water (to make up) were added. Thereafter, 0.2 ml of 0.1% w/v solution of TTC and 0.2 ml of the standardized Cryptococcus sp. and Saprochaete sp. suspensions were added into each tube to obtain varying concentrations of the mixture ratios. The final concentration of the ternary mixtures of Zn, Cd and phenol ranged from 0 to 16 mM. Each concentration of the mixtures was prepared in triplicates. Controls were prepared consisting of the medium without the toxicants. The cultures were incubated at room temperature (28 ± 2^{o}C) for 24 h. After incubation, the extraction and quantification of TTC-formazan were done as described by Nweke et al. [30].

2.5 Data analysis

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The response of the organisms to each concentration of ternary mixtures of phenol, zinc and cadmium was calculated as percent inhibition of dehydrogenase activity (R) relative to the mean control (Eq. 1) [31].

$$R = \frac{C_A - T_A}{C_A} \times 100 \quad (1)$$

Where: R is the inhibition (%) of dehydrogenase activity; C_A is the absorbance of TPF extract in the control. T_A is the absorbance of TPF extract in the test with different concentrations of the toxicants ternary mixtures.

2.6 Determination of Toxicity Threshold

The dose response data from the assessment of toxic effects of the toxicants in their ternary mixtures on dehydrogenase activities of the test organisms were plotted and fitted with 3-parameter logistic function (equation below) [32]

$$R = \frac{100}{1 + \left(\frac{x}{EC_{50}}\right)^b} \quad (2)$$

Where: x is the concentration of the toxicant, EC_{50} is the concentration that caused 50% inhibition; b is parameter determining the relative slope at EC_{50}.

In order to predict hormesis, i.e. stimulation of enzyme activity at low concentration of the mixtures, the dose-response data were fitted to hormetic model equation stated below [33].

$$\text{Inhibition } (\%) = 100 - \frac{100 - fx}{1 + \left[1 + \left\{\frac{2FIC_{50}}{100}\right\}\right]\left(\frac{x}{IC_{50}}\right)^b} \quad (3)$$

Where: f is the parameter describing the degree of hermetic response.

2.7 The toxic index (TI)

The Toxic Index (TI) of each mixture was calculated as the sum of toxic units for all mixture components (Eq.4).

$$TI = \sum_{i=1}^{n} \frac{C_i}{EC_{50i}} = \sum_{i=1}^{n} \frac{\pi_i EC_{50mix}}{EC_{50i}} \quad (4)$$

Where C_i is the concentration of the ith component in the mixture and EC_{50i} is the concentration of the ith component that elicited 50% decrease in dehydrogenase activity when tested as an individual, n is the number of components in the mixture and πi is the proportion of ith component in the mixture. The effect of the mixture is interpreted as antagonism or synergism if TI is greater than 1 or less than 1 respectively. The effect is described as additive if TI equals 1 [33].

III. RESULT

3.1 Toxicity of ternary mixture

Figure 1 showed the effects of ternary mixtures of zinc, cadmium and phenol on Saprochetae sp. dehydrogenase activity. Figure 2 showed the effects of ternary mixtures of zinc, cadmium and phenol on Cryptococcus sp. dehydrogenase activity. The model fit curve for the assessment of the toxic effects of ternary mixtures of zinc, cadmium and phenol on dehydrogenase activity of both Cryptococcus sp. and Saprochetae sp. showed progressive inhibitory effects on the dehydrogenase (enzyme) activity test of Cryptococcus sp. and Saprochetae sp. as the concentration increases. Figure 3 and fig 4 showed the equi-effect of the ternary mixtures of zinc, cadmium and phenol on Saprochetae sp. and Cryptococcus sp. dehydrogenase activities respectively. The result shows that there is an inhibitory effect on the dehydrogenase activity of both Cryptococcus sp and Saprochetae sp as the concentration of the mixtures increases.

The toxicity threshold for the ternary mixtures of the chemicals on the enzyme activity of both Cryptococcus sp. and Saprochaete sp., are shown in Table I. mixture ratio of 50% zinc + 45% phenol +5% cadmium exerted the highest toxicity on both Saprochaete sp. and Cryptococcus sp. with IC_{50} of 0.466 mM and 0.43 mM respectively, while mixture ratio of 27.2% zinc + 71.1% phenol +1.7% cadmium had the lowest toxicity with IC_{50} of 1.151 mM for Saprochaete sp and IC_{50} of 1.215 mM on mixture ratio of 28% zinc + 70% phenol + 2% cadmium for Cryptococcus sp. Toxicity of Equi-Effect (EE50) mixtures was highest on dehydrogenase activity of Cryptococcus sp with IC_{50} of 0.746 mM, while it exerted a low toxic effect on Saprochaete sp with IC_{50} of 1.89 mM

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Toxic interactions of the ternary mixtures of the chemicals on dehydrogenase activity of both Cryptococcus sp. and Saprochaete sp are shown in Table II. All the mixtures ratios showed synergistic interaction for both Saprochaete sp and Cryptococcus sp.

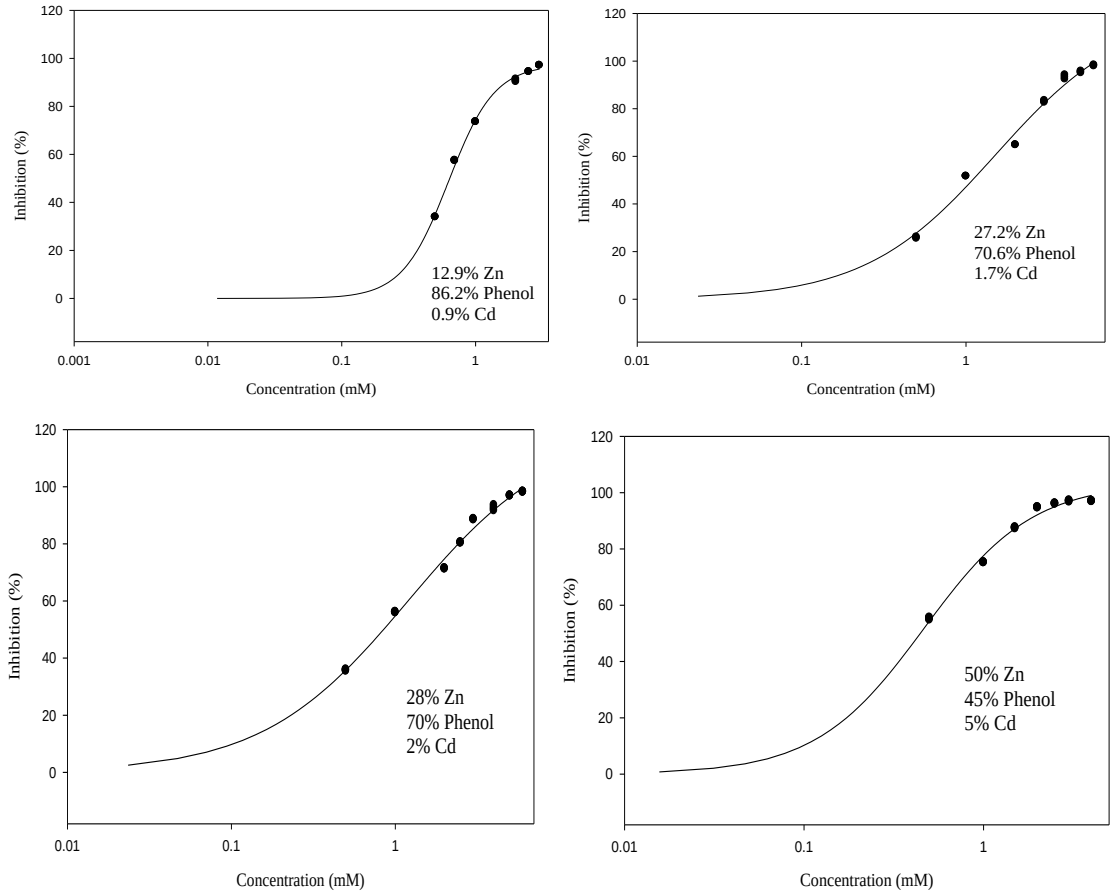

Figure 1: Toxicity of ternary mixtures of zinc, cadmium and phenol on Saprochaete sp.

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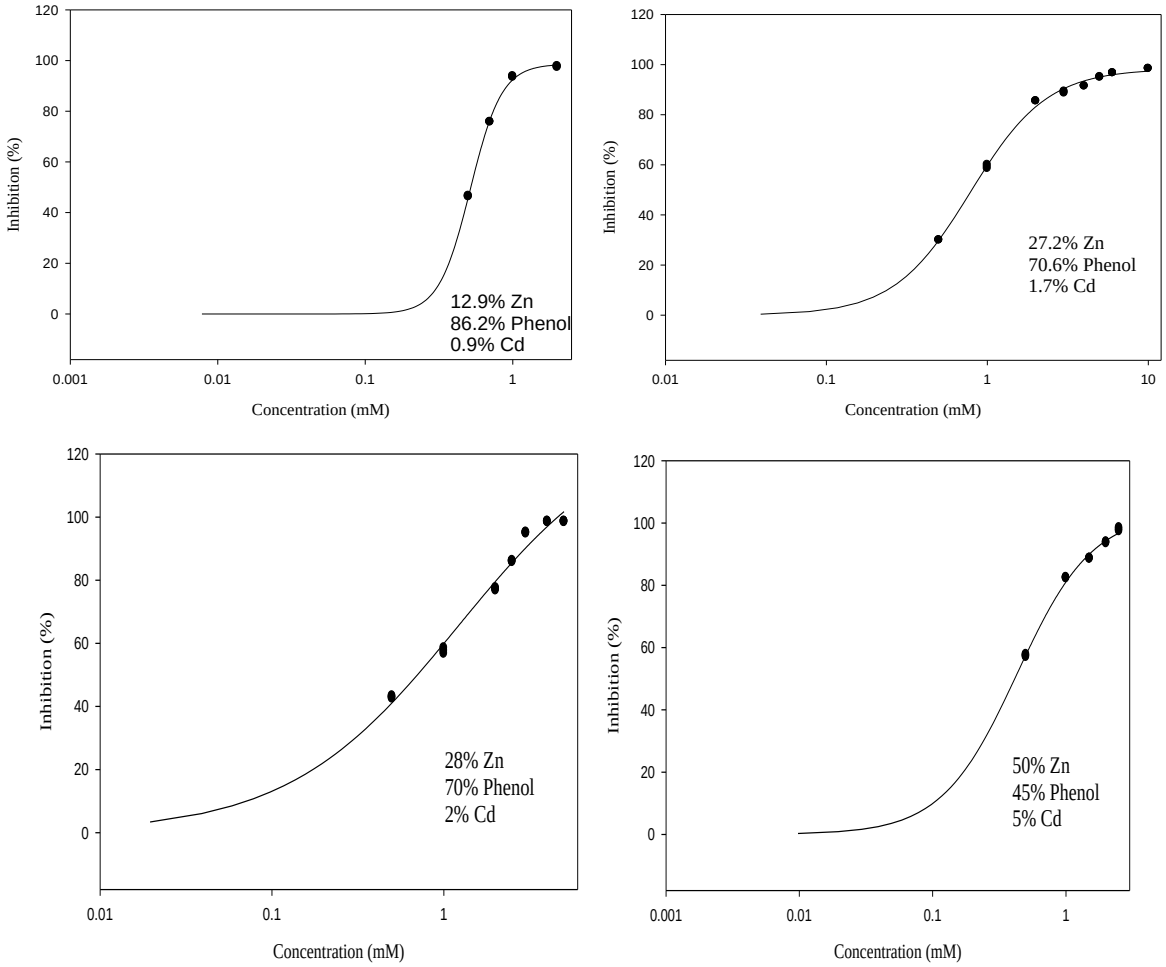

Figure 2: Toxicity of ternary mixtures of zinc, cadmium and phenol on Cryptococcus sp.

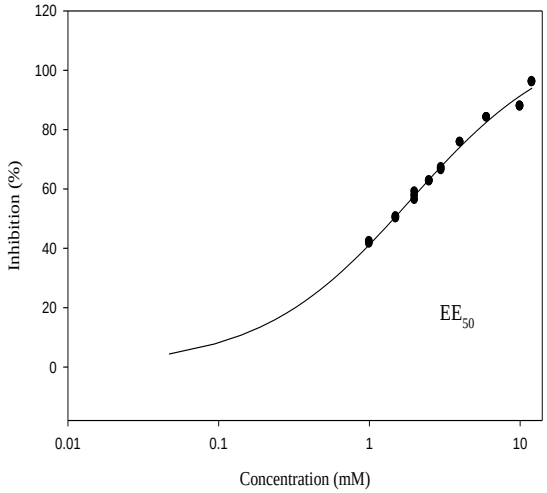

Figure 3: Equieffect (EE_{50}) mixture of Zinc, Phenol and Cadmium on Saprochaete sp.

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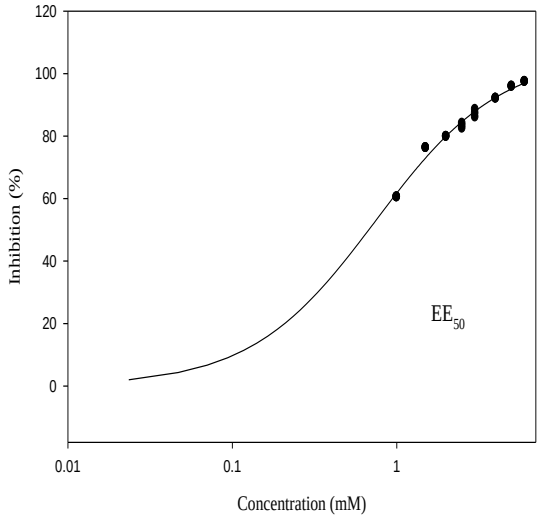

Figure 4: Equieffect (EE_{50}) mixture of Zinc, Phenol and Cadmium on Cryptococcus sp.

Table I: Toxicity thresholds (IC_{50}) of ternary and equi-effects mixtures on dehydrogenase activity of the Cryptococcus sp. and Saprochaeta sp.

	IC_{50}(mM)	
Chemical/Ratio	**Saprochaeta sp.**	**Cryptococcus sp.**
12.9%Zinc +86.2%Phenol + 0.9%Cadmium	0.627±0.014	0.50±0.0186
27.2%Zinc+71.1%Phenol+1.7%Cadmium	1.151±0.289	0.786±0.044
28%Zinc + 70%Phenol + 2%Cadmium	1.12±0.119	1.215±0.370
50%Zinc + 45%Phenol + 5%Cadmium	0.466±0.016	0.43±0.057
EE_{50} Zinc + Cadmium + Phenol	1.89±0.362	0.746±0.074

Table II: Toxic Interactions of the ternary mixtures of the test chemicals on Cryptococcus sp. and Saprochaeta sp.

Mixture/ Ratio	Saprochaete sp.		Cryptococcus sp.	
	Toxic Index (TI)	Interaction	Toxic Index (TI)	Interaction
12.9%Zinc + 86.2%Phenol + 0.9%Cadmium	0.369	Synergistic	0.139	Synergistic
27.2%Zinc + 71.1%Phenol + 1.7%Cadmium	0.607	Synergistic	0.147	Synergistic
28%Zinc + 70%Phenol + 2%Cadmium	0.909	Synergistic	0.834	Synergistic

IV. DISCUSSION

This study evaluates the toxicity of ternary mixtures of phenol, zinc and cadium to Cryptococcus sp. and Saprochaete sp. Zinc is a trace element, it is toxic to microorganisms at elevated concentrations [32]. Cadmium has been reported to inhibit microbial population and enzymes activity in both aquatic and soil environment. For instance, increasing the concentrations of cadmium in soil was reported to gradually decrease the microbial community and enzymes [34] [35]. Phenols are membrane damaging biocides [36] causing loss of cytoplasmic membrane integrity and thus disruption of membrane functions. Research has shown that increased concentration of phenol, zinc and

cadmium in the soil is detrimental to the microbial population, this reflected in this research, where the ternary mixtures of phenol, zinc and cadmium showed a progressive inhibitory effect on the dehydrogenase activates of both Cryptococcus sp. and Saprochaete sp. Similarly, Nwanyanwu et al. [29] reported that increase in the concentrations of zinc, cadmium and phenolic conmpounds progressively inhibited the dehydrogenase activity of bacterial consortium. Ventura et al. [37] in their work "toxicity of metal cations and phenolic compounds to the bioluminescent fungus Neonothopanus gardneri" also reported that phenol and cadmium were toxic to the fungus Neonothopanus gardneri. Nlemolisa, et al. [28], also reported inhibition of the dehydrogenase activities of yeast by zinc, cadmium and phenol, both as an individual chemical and their binary mixtures. Nweke and Okpokwasili [38] reported inhibition of the dehydrogenase activity of Rhizobium species by glyphosate, 4-Chlorophenol and 2, 4 dichlorophenol and Nweke et al. [39] where glyphosate, phenol, 4-CP and 2, 4-DCP progressively inhibited dehydrogenase activity.

For the toxicity threshold (IC_{50}), mixture ratio of 12.9% zinc +86.2% phenol +0.9% cadmium has an IC_{50} of 1.151 mM (Saprochaete sp) and 0.786 mM (Cryptococcus sp), 27.2% zinc + 71.1% phenol +1.7% cadmium has an IC_{50} of 0.627 mM (Saprochaete sp) and 0.50 mM (Cryptococcus sp), 28% zinc + 70% phenol + 2% cadmium has an IC_{50} of 1.12 mM (Saprochaete sp) and 1.215 mM (Cryptococcus sp) and 50% zinc + 45% phenol +5% cadmium has an IC_{50} of 0.466 mM (Saprochaete sp) and 0.43 mM (Cryptococcus sp). Mixture ratio of 50% zinc + 45% phenol +5% cadmium exerted the highest toxicity on both Saprochaete sp and Cryptococcus sp with IC_{50} of 0.466 mM and 0.43 mM respectively. This highest toxicity exhibited by this mixture 50% zinc + 45% phenol +5% cadmium where cadmium is the highest among the four mixtures shows that cadmium has no physiological function and strongly inhibit microbial metabolism even at low concentrations [32]. This is in agreement with Ventura et al. [37], where cadmium exerted the highest toxicity on Neonothopanus gardner. Cadmium exhibited sharp inhibitory effect on the dehydrogenase enzyme activities of yeasts [28].

Toxic effects includes the blocking of functional groups, displacement and substitution of essential metal ions for biomolecules, conformational modification, denaturation and inactivation of enzymes and distruptions of cellular and organellar membrane integrity Nweke and Okpokwasili [40]. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. For example, lead (Hg2+), cadmium (Cd2+) and silver (Ag2+) tend to bind to sulfhydryl (−SH) groups of enzymes essential for microbial metabolism, and thus inhibit the activity of sensitive enzymes. It can also be due to its chemical similarity to certain essential mineral elements, example zinc (Zn), iron (Fe) and calcium (Ca). Cadmium toxicity rises from displacement of these essential elements from a number of essential metalloproteins.

The Toxic Index values obtained for all the ternary mixtures are less than 1, thus describing synergistic interactions [33].

V. CONCLUSION

Inhibition of dehydrogenase activity was used in assessing the toxic effects of ternary mixtures of phenol, zinc and cadmium on Cryptococcus sp. and Saprochaete sp. The results of this work indicated that the ternary mixtures of phenol, zinc and cadmium, exhibited synergistic interactive effect on the organisms. This study could constitute base line information towards assessing the possible environmental hazards associated with contamination of the environment with phenol, zinc and cadmium.

The less common use of soil microorganisms than that of aquatic microorganisms to assess contaminant toxicity and the poor accuracy of different bioassays strengthens the argument for the need to extend studies to terrestrial species in order to better understand their development and biological responses. Fungi are of special interest in this respect because of their key role in soil environment and their strategic position at the base of the terrestrial food chain.

REFERENCE

[1]. Khan, F.U., Rahman, A.U.,Jan, A. and Riaz, M. (2004). Toxic and trace metals (Pb,Cd,Zn, Cu, Mn, Ni, Co and Cr) in dust, dustfall/soil, Journal of the Chemical Society of Pakistan, **26**(4): 453–456.

[2]. Ali, H., Khan, E. and Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Journal of Chemistry, 1-14.

[3]. Okolo, V.N., Olowolafe, E.A., Akawu, I. and Okoduwa, S.I.R. (2016). Effects of industrial effluents 581 on soil resource in

challawa industrial area, Journal of Global Ecology and Environment, **5**(1):10.

[4]. Siddiquee, S., Rovina, K. and Azad, S.A. (2015). Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review, Journal of Microbial and Biochemical Technology, **7**(6): 384–393.

[5]. Hrynkiewicz, K. and Baum, C. (2014). Application of microorganisms in bioremediation of environment from heavy metals, Environmental Deterioration and Human Health: Natural and Anthropogenic Determinants, 215–227.

[6]. Gautam, R.K., Soni, S. and Chattopadhyaya, M.C. (2014). Functionalized magnetic nano particles for environmental remediation, Handbook of Research on Diverse Applications of Nanotechnology in Biomedicine, Chemistry, and Engineering, 518–551.

[7]. Lakherwal, D. (2014). Adsorption of heavy metals: a review, International Journal of Environmental Research Development, **4**: 41–48.

[8]. Turpeinen, R., Kairesalo, T. and Haggblom, M. (2002). Microbial activity community structure in arsenic, chromium and copper contaminated soils, Journal of Environmental Microbiology, **35**(6): 998–1002.

[9]. Shahid, M., Javed, M.T., Mushtaq, A., Akram, M.S., Mahmood, F., Ahmed, T., Noman, M. and Azeem, M. (2019). Microbe-Mediated Mitigation of Cadmium Toxicity in Plants. In Cadmium Toxicity and Tolerance in Plants; Elsevier: Amsterdam, the Netherlands, 427-449.

[10]. Tran, T.A. and Popova, L.P. (2013). Functions and toxicity of cadmium in plants: Recent advances and future prospects. Turkish Journal of Botany, **37**:1–13.

[11]. Dugar, G., Herbig, A., Förstner, K.U., Heidrich, N., Reinhardt, R., Nieselt, K.and Sharma, C.M. (2013). High-Resolution Transcriptome Maps Reveal Strain-Specific Regulatory Features of Multiple Campylobacter jejuni Isolates. PLOS Genetics, 9.

[12]. Rasmussen, L.D., Sørensen, S.J., and Turner, R.R.and Barkay, T. (2000). Application of a mer-lux biosensor for estimating bioavailable mercury in soil, Soil Biology and Biochemistry, **32**(5): 639–646.

[13]. Gauthier, P.T., Norwood, W.P., Prepas, E.E. and Pyle, G.G. (2014). Metal-PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes, Aquatic Toxicology, **154**: 253–269.

[14]. Hildebrandt, U., Regvar, M. and Bothe, H. (2007). Arbuscular mycorrhiza and heavy metal tolerance, Phytochemistry, **68**(1): 139–146.

[15]. Agency for Toxic Substances and Disease Registry (ATSDR) (2008). Toxicological profile for phenol. Division of toxicology and environmental medicine/applied toxicology branch US Department of health and human services. Georgia, United States, 1–20.

[16]. Busca, G., Berardinelli, S., Resini, C. and Arrighi, L. (2008). Technologies for the removal of phenol from fluid streams: a short review of recent developments. Journal of Hazard Materials, **160**: 265–288.

[17]. Wasi, S., Tabrez, S. and Ahmad, M. (2013). Toxicological effect of major environmental pollutants: An overview. Environmental Monitoring and Assessment, **185**: 2585–2593.

[18]. Gayathri, K.V. and Vasudevan, N. (2010). Enrichment of phenol degrading moderately halophilic bacterial consortium from saline environment. Journal of Bioremediation Biodegradation, **1**(1): 104–110.

[19]. Karci, A. (2014). Review: Degradation of chlorophenols and alkylphenol ethoxylates, two representative textile chemicals, in water by advanced oxidation processes: the state of the art on transformation products and toxicity. Chemosphere, **99**: 1–18.

[20]. Moridani, M.Y., Sirakia, A., Chevaldinat, T., Scobie, H. and Obrien, P.J. (2004). Quantitative structure toxicity relationship for catechols in isolated rat hepatocytes. Chemico-Biological Interactactions, **147**: 297–307.

[21]. Marrot, B., Barrios-Martinez, A., Moulin, P. and Roche, N. (2006). Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochemical Engineering Journal, 174–183.

[22]. Barlow, J., Johnson, J.A.P., Belcher, S.M. and Fenton, S.E. (2007). Early life exposure to phenols and breast cancer risk in later years: Fact sheet on phenols. Breast Cancer and the Environment Research Centre Community Outreach and Translation Cores, 1–20.

[23]. Luo, Q., Wang, H., Zhang, X., Fan, X. and Qian, Y. (2006). In situ bioelectrokinetic

remediation of phenol-contaminated soil by use of an electrode matrix and a rotational operation mode. Chemosphere, **64**: 415–422.

[24]. Boudet, A.M. (2007). Evolution and current status of research in phenolic compounds: Review. Phytochemistry, **68**: 2722–2735.

[25]. Cordova-Rosa, S.M., Dams, R.I., Cordova-Rosa, E.V., Radetski, M.R., Corrêa, A.X. and Radetski, C.M. (2009). Remediation of phenol-contaminated soil by a bacterial consortium and Acinetobacter calcoaceticus isolated from an industrial wastewater. Journal of Hazardous Materials, **164**: 61–66.

[26]. Chakraborty, S., Bhattacharya, T., Patel, T.N. and Tiwari, K.K. (2010). Biodegradation of phenol by native microorganisms isolated from coke processing wastewater. Journal of Environmental Biology, **31**: 293–296.

[27]. Nwanyannwu, C.E. and Abu, G.O. (2013). Biodegradation of phenol at low and high doses by bacterial strains indigenous to Okrika River in the Niger Delta of Nigeria. Journal of Biological Research, **3**(3): 911–921.

[28]. Nlemolisa, O.R., Nwanyanwu, C.E., Akujobi, C.O., Ihenetu, F.C., Nwokorie, R.C., Obasi, C.C., Kemka, U.N., Uzoho, K.H. and Nwoke, M.C. (2020). Toxicity of binary mixtures of phenol, zinc and cadmium to yeast strains isolated from hydrocarbon impacted soil. Open Access Library Journal, **7**: 1-15.

[29]. Nwanyanwu, C.E., Adieze, I.E.,Nweke, C.O. and Nzeh, B.C. (2017). Combined effects of metals and chlorophenols on dehydrogenase activity of bacterial consortium. International Research Journal of Biological Sciences, **6**(4):10-20

[30]. Nweke C. O., Ike C. C. and Ibegbulem C. O. (2016). Toxicity of quaternary mixtures of phenolic compounds and formulated glyphosate to microbial community of river water. Ecotoxicology and Environmental Contamination, **11(**1): 63 – 71.

[31]. Okechi, R.N., Chukwura, E.I. and Nweke, C.O. (2020). Predicting the toxicities of ternary mixtures metals and Sodium Dodecyl sulphate to Serratia marcescens (SerEW01) from Otamiri River Water. Journal of Advances Microbiology, **20**(9): 73-86.

[32]. Nweke. C.O., Umeh, S.I. and Ohale, V.K. (2018). Toxicity of four metals and their mixtures to Pseudomonas fluorescens: An assessment using fixed ratio ray design. Ecotoxicology and Environmental Contamination, **13**(1): 1-14

[33]. Boillot, C. and Perrodin, Y. (2008). Joint-Action Ecotoxicity of Binary Mixtures of Glutaraldehyde and Surfactants Used in Hospitals: Use of the Toxicity Index Model and Isobologram Representation. Ecotoxicology and Environmental Safety, **71**: 252-259.

[34]. Abdousalam, A.G. (2010). Effect of heavy metals on soil microbial processes and population. Egyptian Academic Journal of Biological Science, **2**(2):9-14.

[35]. Xiao, L., Yu, Z., Liu, H., Tan, T., Yao, J., Zhang, Y. and Wu, J. (2020). Effects of Cd and Pb on diversity of microbial community and enzyme activity in soil. Ecotoxicology, **29**(5):551-558.

[36]. Okolo, J. C., Nweke C. O., Nwabueze R. N., Dike C. U. and Nwanyanwu C. E. (2007). Toxicity of phenolic compounds to oxidoreductases of Acinetobacter species isolated from a tropical soil. Scientific Research Essay, **2**:244–250.

[37]. Ventura, F.F., Soares, D.M.M., Bayle, K., Oliveira, A.G., Bechara, E.J.H., Freire, R.S. and Stevani, C.V. (2021). Toxicity of metal cations and phenolic compounds to the bioluminescent fungus Neonothopanus gardneri, Journal of Environmental Advances, **4**- 1:7.

[38]. Nweke, C.O. and Okpokwasili, G.C. (2014). Kinetics of growth and phenol degradation by Pseudomonas species isolated from petroleum refinery wastewater. International Journal of Bioscience (IJB), **4**(7):28-37.

[39]. Nweke, C.O., Ike, C.C. and Ibegbulem, C.O. (2016). Toxicity of quaternary mixtures of phenolic compounds and formulated glyphosate to microbial community of river water. Ecotoxicoloy and Environmental Contamination, **11**(1): 63-71.

[40]. Nweke, C.O. and Okpokwasili, G.C. (2010). Influence of exposure time on phenol toxicity to refinery wastewater bacteria. Journal of Environmental Chemical and Ecotoxicology**, 2**: 20-27.