TOXICITY OF HEAVY METALS AND SODIUM DODECYL SULPHATE MIXTURES TO ENVIRONMENTAL BACTERIAL ISOLATES FROM OTAMIRI RIVER, IN IMO STATE

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING, FACULTY OF BIOSCIENCES, NNAMDI AZIKIWE UNIVERSITY, AWKA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (PhD) DEGREE IN ENVIRONMENTAL MICROBIOLOGY

MAY, 2021

CERTIFICATION

I, OKECHI, REUBEN NWOYE with registration number NAU/PG/PhD/2013487001F have satisfactorily completed the requirements for course and research work, for the award of a Doctor of Philosophy (PhD) in Environmental Microbiology, from the Department of Applied Microbiology and Brewing. The research work is original and has not been submitted in part or full for any other diploma or degree of this or any other university, to the best of my knowledge.

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APPROVAL PAGE

This dissertation titled "Toxicity of heavy metals and sodium dodecyl sulphate mixtures to environmental bacterial isolates from Otamiri river, in Imo State" carried out by Okechi, Reuben Nwoye (NAU/PG/PhD/2013487001F) has been examined and approved for the award of the degree of Doctor of Philosophy (PhD), in Environmental Microbiology of the Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka.

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This research work is dedicated to my late parents, Chief and Lolo N Okechi

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ABSTRACT

Otamiri is one of the major rivers that passes through Owerri urban and its environs. It serves as a source of aquatic food and water for domestic activities, irrigation among others. All the drainages discharge their untreated waste waters into this river. The study assessed the toxicity of heavy metals and sodium dodecyl sulphate (SDS) mixtures to environmental bacterial isolates from Otamiri river water and sediment. Physicochemical parameters of the river water and sediment were analysed using soxhlet extraction, atomic absorption spectrometry, and gas chromatography. Standard microbial techniques such as serial dilution, spread plate culturing techniques, plate counts, morphological and biochemical characterization were used in determining the preponderant bacterial isolates from the river and its sediment. The identities of the preponderant isolates were further confirmed using 16S rRNA gene partial sequencing and were subsequently adopted for the toxicity assay. The toxicities of the heavy metal ions, Pb(II), Cd(II), Ni(II), Zn(II) and Co(II), as individuals, and in binary, ternary, quaternary, quinary and senary mixtures with SDS against the preponderant bacteria from the river water and sediment were assessed, using inhibition of dehydrogenase activity as the response. Similarly, fixed ratio design [Arbitrary concentration ratio (ABCR) and EC_{50} equieffect concentration ratio (EECR50)]was employed in evaluating the toxicities of the mixtures to the preponderant bacteria. The effects of the mixtures on the dehydrogenase activity were assessed using toxic index, model deviation ratio and isobolographic analyses. In addition, the toxicities of the mixtures were predicted with concentration addition (CA) and independent action (IA) models. The experimentally-derivedEC_{50S} for each toxicants as well as for the four-mixture ratios in each mixture type were compared. Similarly, within each mixture ratio, the experimentally-derived EC_{50}, CA- and IA-models predicted EC_{50S} were equally compared using Duncan post-hoc tests, implemented with SPSS Statistics 21 at $P<0.05$.In Otamiri river water,iron(Fe) recorded the highest value among the heavy metals (1.972 mg/l), followed by zinc (Zn) (1.556 mg/l), while cobalt (Co) was not detected. Similarly, lead (Pb), cadmium (Cd), nickel (Ni), mercury (Hg), conductivity and turbidity recorded values higher than WHO recommended quality standards for drinking water. In the sediment, Fe and Cd recorded the highest and least values 19.82 and 0.025 mg/kg respectively. The pH of the river and sediment were 6.42 and 5.40. Similarly, SDS was the predominant anionic surfactant in both the river water (0.100 µg/l) and sediment (0.453 µg/kg), while perfluorobutane sulfate was not detected in the river water. The bacteriological analysis showed the presence of *Serratia marcescens* (SerEW01) (33.33%), *Staphylococcus* (22.20%), *Streptococcus* (22.20%), *Enterobacter* (11.11%), *Escherichia* coli (11.11%) as well as *Acinetobacterseifertii* (42.10%), *Bacillus* (15.80%), *Escherichia* coli (15.80%), *Klebsiella* (10.53%) and *Streptococcus* species (5.30%), in the river water and sediment respectively, with their percentage occurrences. The responses of both bacteria to the inhibitory effects of the individual toxicants and their various mixtures were concentration-dependent, increasing progressively as the concentrations increased.All the dose-response relationships of the ABCR and EECR50 mixtures and the individual toxicantswere described by logistic function.The experimental EC_{50S} ranged from 0.046 ± 0.003 mM (Zn(II)) to 2.329 ± 0.092 mM (SDS) against*S. marcescens* (SerEW01) as well as from 0.011 ± 0.000 mM (Cd(II)) to 2.810 ± 0.140 mM (SDS) against*A.seifertii*.Duncan tests for both bacteria indicated that the EC_{50S} of the individual toxicants differed significantly from oneanother and the order of decreasing toxicities were Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS for *S. marcescens* (SerEW01) and Cd(II) > Co(II) > Zn(II) > Pb(II) > Ni(II) > SDS for *A.seifertii*.In

binary mixtures of SDS+metal ion against *S. marcescens* (SerEW01), SDS 98.08% + Co(II) 1.92% mixture ratio was hormetic, while CA and IA models predicted similar toxicities in SDS+Ni(II) binary mixtures. In the binary mixtures of SDS+metal ion against *A. seifertii*, SDS+Co(II) and ABCR3 mixture ratio of SDS+Cd(II) mixture type showed no statistical differences between CA and IA-model predicted EC_{50S}. SDS+Zn(II) binary mixtures were also hormetic at low concentrations. The CA and IA models underestimated the binary mixture toxicities against both organisms. All the ternary mixtures of SDS and two metals were very toxic against *S. marcescens* (SerEW01), even at low concentration, with EC_{50S} raging from 0.102 ± 0.006 mM to 0.203 ± 0.009 mM. There was no significant difference between CA and IA-models predicted EC_{50S} in ABCR1 and ABCR3 mixture ratios of SDS+Ni(II)+Cd(II) and SDS+Co(II)+Cd(II) ternary mixtures respectively, against *A.seifertii*. In ABCR1 mixture ratio of SDS+Ni(II)+Cd(II) ternary mixture, both models almost correctly predicted the experimentally-derived data, while the models overestimated the mixture toxicities in the other ternary mixtures. In all quaternary mixtures, both models predicted lower toxicities compared to the experimentally-derived data against *S. marcescens* (SerEW01). Similarly, the CA model correctly predicted the experimentally-derived data at low concentrations in SDS+Cd(II)+Zn(II)+Pb(II) quaternary mixtures against *A.seifertii*. In quinary mixtures, ABCR2 mixture ratio of SDS+Cd(II)+Zn(II)+Pb(II)+Co(II) mixture was stimulatory against *A.seifertii* at low concentrations and both models underestimated the interactive effects of the mixtures on both bacteria. Similarly, senary mixtures of SDS+five metal ions were also toxic against both organisms even at low concentrations. In most mixtures, the interactive effect was strongly synergistic against both bacteria. Otamiri river water and sediment were contaminated by heavy metals and sodium dodecyl sulfate. Some of these heavy metals and SDS inhibited the dehydrogenase activities in the preponderant bacteria from the river water and sediment, both as individual toxicants and their mixtures. The mostly synergistic effect reported in mixtures in this study demonstrates the potential danger of co-contamination of the aquatic ecosystems by SDS and heavy metals.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

An unlimited number of different mixtures of pollutants occur in the environment, and the number and concentration of chemicals in these mixtures are variable (Ishaque *et al.*, 2006). In everyday life, humans and other living organisms are rarely exposed to single stressors, but to a mixture of different stressors; either concurrently, sequentially, or both (Prince *et al*.,2002; Moser *et al*., 2005; Lokke*et al*., 2012).Chemical pollution of the environment by surfactants and heavy metal ions is as a result of increasing industrial activity of man. Large amounts of these pollutants when penetrating into surface water reservoirs cause foaming, reduced diffusion of the atmospheric oxygen dissolved in water and consequently lead to the death of many organisms due to deficiencies of oxygen (Seifert and Domka, 2005). The toxicity of chemical compounds on aquatic organisms depends on concentration in both sediments and the water, as well as in processes related to their bioavailability. Bioaccumulation, biodegradation, desorption and solubilization processes that occur in these substrata determine the quantity of free compounds that will reach toxic levels in the organs of aquatic organisms (Flores *et al*., 2010).

According to Weast (1984), heavy metals are metals with a density above 5 g/cm^3, thus, of the 90 naturally occurring elements, 53 are heavy metals.Apart from natural sources, some of the anthropogenic activities that have contributed to heavy metals contamination of the environment include automobile emissions, mining activities, battery industry, fossil fuels, metal plating, electronic industries, oil and petrochemicals spillage, as well as variousagricultural practices (Khan*et al*.,2008;Zhang*et al.*, 2010;Chaturvedi and Tiwari, 2013).

Sodium dodecyl sulfate (SDS), an anionic surfactant, is the most widely used synthetic organic chemical found in detergents, shampoos, cosmetics, household cleaners, herbicides, and dispersants used in oil-spill cleanups (Cowan-Ellsherry *et al*., 2014). The major exposure route for SDSto aquatic environments is through contaminated waters, sediments, or soils, which threatens drinking water supplies or organisms living in these environments (Singer and Tjeerdema, 1993).

Many researchers have established the toxicity of heavy metal mixtures to living organisms at low or high concentrations (Gikas, *et al.,* 2009; Nweke and Okpokwasili, 2011; Rathnayake*et al.,* 2013; Kouchou *et al*., 2017; Nweke*et al.,* 2018), and there has also been reports on the toxicity ofsome chemical surfactants by some authors (Ying, 2006; Chaturvedi and Tiwari, 2013; Yuan *et al*., 2014;Effendi *et al.,* 2017), whereas others reported anionic surfactants as being safe. It has been established that some anionic surfactants can enhance the toxicity of coexisting chemical species, such as metals, and anthracene (Swedmark and Granmo, 1981; Flores *et al*., 2010).Adverse health effects have been demonstrated from exposure to multiple chemicals at low concentrations, which individually would not cause harm (Brian *et al.,* 2007; Smith *et al*., 2013;Kortenkamp, 2014). In concentrations higher than the threshold of toxicity, the surfactants and heavy metal ions often lead to inhibition of the process of self-purification of ground waters and soil and delay self-sustaining processes in the environment (Seifert and Domka, 2005).

Microorganisms are vital for the efficient functioning of any ecosystem; hence factor that affect their metabolism, composition and abundance are of great concern. Monitoring microbial responses has been recommended as an early warning indicator of ecosystem stress, as microbes respond promptly to environmental perturbations (Griffiths, 1983; Odum, 1985).

Though much work has been done on the toxicity of heavy metals and their mixtures to microorganisms in the environment(Nweke *et al*., 2018; Chu, 2018; Osigwe *et al*., 2020; Nweke *et al*., 2020) and some on anionic surfactants**,** only few researchers have looked at the toxicities of the mixtures of metals and sodium dodecylsulfate (SDS)on microbes in the environment.

1.2 Statement of the Problems

Otamiri river is the major river that passes through Owerri urban and its environs. This river serves as a source of aquatic foods and water for domestic activities, urban agriculture and other purposes. All the drainages in Owerri urban and its environs discharge their untreated wastewaters into the river or its tributary, Nworie river. Nekede automechanic village, hospitals, car washing and laundry outfits are located along the banks of the river. When it rains, run-offs from Owerri urban and environs gain unrestricted access into the river. In addition, sand mining activities that go on in the river(Plate i) tend to suspend and redistribute dissolved organic and inorganic chemical substances in the river. Solid wastes are also deposited in dumps and incinerated at the river bank(Plate ii).These waste dumps contain a wide variety of chemical substances that leach into the river. These activities have contributed to the high-level of heavy metals and anionic surfactants contamination of the river and its sediment. These chemical toxicants may have some deleterious effects on the microbes in the riverwater and its sediment.

1.3Aim of the Study

This work aims at determining the toxicity of some heavy metals and sodium dodecyl sulphate andtheir mixtures to the preponderant bacterial isolates from Otamiri river water and sediment.

1.4.Objectives of the Study

The specific objectives of the study are to determine the:

i. physicochemical characteristics of Otamiri river water and sediment.

ii. types and levels of heavy metals contamination of the river water and sediment.

iii. types and levels of anionic surfactants contamination of the river water and sediment.

iv. preponderant bacterial isolates from Otamiri river water and sediment.

v. toxicities of some of the identified heavy metals andan anionic surfactant (sodium dodecyl sulfate(SDS),as well as their mixtures to the most prevalent bacterial isolates from the river water and sediment.

vi. interactive effects of metal ions+SDS mixtures to bacterial dehydrogenase activity using predictive mathematical models.

1.5. Rationale for the Study

Many studies have been carried out on the heavy metals and bacteriological contamination of Otamiri river water and sediment. However, there has been no published work on the anionic surfactants content as well as the possible toxic effects of the mixtures of these toxicants on the aquatic bacterial flora of both the river water and sediment. Thus, this work opens a new perspective in pollution studies on the river and its sediment.

1.6. Significance of the Study

This study is significant in view of the various uses that Otamiri water resources are put to, such as domestic and agricultural purposes. The hazardous effects associated with the toxicants and their mixtures on the aquatic lifes in the river, vegetables and plants irrigated with the river water, as well as on the people consuming these foods and drinking the river water, could be enormous. The outcome of this study could, therefore, be beneficial to Government ministries, Departments and Agencies, as well as the general public.

1.7. Scope of the Study

This study covers the bacteriological and physicochemical qualities, including the heavy metals and anionic surfactant contents of Otamiri river water and sediment. The study also determined the toxicity of some of these predominant heavy metals and an anionic surfactant from both environmental media, as well as their various mixtures to the preponderant bacterium from each medium.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.Heavy Metals

Heavy metal contamination is a major environmental problem because of their non degradability and toxicity. Heavy metals are threat to human life and environment. Much research has been conducted on heavy metals contamination in soil from various sources such as industrial wastes, automobile emission and agricultural practices. According to Gurave *et al.*,(2015) andIshaque *et al*., (2006),metals are classified into three categories on the basis of their biological function and effects: (1) the essential metals with known biological function. These include the following: Na, K, Mg, Fe, Co, Ni, Cu and Zn. (2) the toxic metals such as Ag, Cd, Sn, Au, Hg, Ti, Pb, Al, Ge, As, Sb and Se. (3) the non essential non toxic metals with no known biological effects, these include: Rb, Cs, Sr (Gurave *et al.*, 2015). Heavy metals are classified as (i) bound to reducible phases (ii) exchangeable (iii) bound to organic matter and sulphides. Excessive levels of heavy metals like zinc, cadmium, copper, lead, nickel and mercury are considered as mainly toxic pollutants (Xie*et al.*, 2010). Lead, a major pollutant that is found in atmosphere is greatly toxic to humans, animals, plants and microorganisms. Heavy metals contaminate the environment by gathering in food chain and remains in nature. Each heavy metal has its own toxicity. Copper and zinc can improve microbial growth at low concentrations but suppresses growth at elevated concentrations (Smejkalova *et al.*,2003; Igwe *et al.*,2005; Hookoom and Puchooa, 2013;).

2.2.Toxicity of Heavy Metals to Microorganisms

Although most researches with non-essential metals are carried out with individual metals, in reality, organisms are often exposed to multiple contaminants at the same time through the air, food and water. Heavy metals exert a significant effect on soil microbes and soil processes, thus disturbing the biological equilibrium of soil, followed by soil degradation (Huang and Shindo, 2000). Studies have shown that long-term heavy metal contamination of soils has harmful effects on soil microbial activity, especially microbial respiration (Doelman and Haanstra, 1984). Apart from long-term metal-mediated changes in soil enzyme activities, many reports have shown large reductions in microbial activity due to short-term exposureto toxic metals (Hemida *et al.*,1997). Also, Babich and Stotzky (1977), demonstrated that heavy metals are highly toxic to soil microbes.

The impact of heavy metals on microorganisms and on enzymatic activity depends, among others, on soil pH, content of organic and mineral colloids, as well as on the type of heavy metals and their chemical properties (Kucharski and Wyszkowska, 2004).In a study to assess the individual and combined toxicities of four non-essential metals (As, Cd, Hg and Pb) in microtox assay using *Vibriofisheri*, among the individual metals tested, the toxicity ranking based on *EC50* was Hg>Pb>Cd>As and among metal mixtures, synergism was evident (Ishaque *et al*., 2006). Utgikar *et al*.,(2004), also in their study on "toxicity of metals and metal mixtures,which relies upon the attenuation of light intensity emitted by *Vibriofisher*, observed that the toxic effect of zinc asymptotically approached a maximum with respect to concentration at all times, while that of copper increased exponentially with concentration without any limiting maximum value. They also noted that the toxic effects of binary mixtures were substantially higher than those expected on the basis of additivity of individual metals.

In a study on the "effect of heavy metals on soil microbial community and mung beans seed germination", Ashraf and Ali (2007), reported that lead and silver were found to be toxic to the growth of microorganisms, while zinc at 50mM concentration facilitated the growth of bacteria and fungi. However, there was an overall change in the microbial communities compared to the control.Interactions between nickel and other heavy metals was observed to reduce the population size of *Azotobacter* species and other bacteria, actinomyces and fungi in study by Wyszkowska *et al.*,(2007). In their study on "toxicity of heavy metals to microbial community of new Calabar river", Hg, Cd, Zn, Cr, Ni and Cu were reported to adversely affect the metabolic activities of the microbial community (planktonic and sediment community) of New Calabar River (Nweke and Orji, 2009).

2.3. Toxicity of Heavy Metals to Microbial Enzymes

Heavy metals have also been reported to affect microbial enzymes significantly.The inhibition of soil enzyme activities by heavy metals is a very complex issue, as there are many factors that affect this inhibition. These factors can be divided into four main classes: metal factors, enzyme factors, soil factors, and plant factors. Metal factors include the heavy metal element in question, the concentration of the heavy metal, the chemical form of the heavy metal, the availability of the heavy metal, and indirect effects of the heavy metal. Enzyme factors include the enzyme sensitivity, the structural inhibition of the enzyme, and the major properties of the enzyme. Soil factors include pH, organic matter, and clay. Finally, plant factors include metal accumulation and plant community effects (Karoca *et al.*,2010).

Generally, toxic metals cause enzyme inactivation, damaging cells by acting as antimetabolites or form precipitates or chelates with essential metabolites. Enzyme activity is a soil property that is chemical in nature but has a direct biological origin. This activity arises from the

presence of many types of enzymes that are present in the soil, and within soil microorganisms.Phosphates have been reported to be sensitive to heavy metals such as cadmium, chromium, copper and zinc (Doelman and Haanstra, 1989). In their study, Nweke and Okpokwasili (2011), reported the repression of α-glucosidase and β-galactosidase induction in *Escherichia coli, Bacillus and Pseudomonas* species isolated from petroleum refinery effluent by zinc and cadmium.Similarly, in a study on toxicities of senary and septenary mixtures of five metals and two phenols to *Pseudomonas fluorescens*, nikel, cobalt, zinc, cadmium, lead were reported to inhibit dehydrogenase activity in the soil bacterium (Nweke *et al*., 2020). It is well established that toxic effects of heavy metals are highly selective in the higher organisms. Specific organ targeting was shown for mercury and silver in invertebrates (Bianchini *et al.,*2005; Inza *et al*., 2004). Indications of specific inhibitory action ofheavy metals have been produced in microbes as well (Fulladosa *et al.,* 2005a, b). Such selective targeting of specific enzymaticsystems and pathways suggests that certain members ofthe microbial community would be more sensitive toheavymetal exposure than others, depending on thesensitivity of their critical metabolic pathways. Thus,while toxicity of heavy metals to microbes is a wellestablished phenomenon, the effects of those metals uponspecific enzymatic systems at lower ("sub-acute")concentrations are not well known (Sobolev and Begonia, 2008).

2.4. Mechanisms of Microbial Resistance to Heavy Metals

Microorganisms continued existence in polluted soils depends on intrinsic biochemical and structural properties, genetic and physiological adaptation including morphological changes of cells, as well as ecological modifications of metal speciation. To endure under metal-stressed circumstances, bacteria have evolved up to several types of adjustment to stand the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell,

bioaccumulation of the metal ions inside the cell, and the decreasing concentration of the heavy metal ions (Gadd, 1990). Also, some bacteria have adapted to heavy metal contaminated environment through a range of plasmid-mediated resistance systems (Silver and Misra, 1988).Metal tolerance of bacteria could depend upon several factors, namely types of metal ion transport into the cell, localization of metal resistance genes (chromosome, plasmid or transposon), and the role of metal ion in the cellular metabolism as reported by Bruins et al.,(2000). AccordingtoBruins *etal.* (2000) andChoudhury and Srivastava (2001),there are five main mechanisms of heavy metal resistance of bacteria and it should be noted that the same bacterium could possess several protection mechanisms:

- extracellular barrier;
- active transport of metal ions (efflux);
- extracellular sequestration;
- intracellular sequestration;
- reduction of metal ions.

2.4.1. Extracellular barrier as a way of preventing metal entry into the cell

The cell wall, plasma membrane or capsule could prevent metal ions from entering the cell. Bacteria belonging to different taxonomical groups can adsorb metal ions by ionazable groups of the cell wall or capsule (carboxyl, amino, phosphate and hydroxyl groups) (El-Helow*et al.*,2000; Taniguchi *et al.,* 2000). This adsorption is a passive process as dead bacterial cells are also capable of binding metal ions (Pardo *et al.*,2003). Bacterial cells killed by thermal treatment have been shown to possess the same or even higher adsorption capacity as the living cells (Yilmaz, 2003). For example, high level of passive sorption of heavy metal ions was observed for non-viable cells of *Pseudomonasputida,Brevibacterium*species

and*Bacillus*species(Green-Ruiz, 2006). Several authors observed that metal ion accumulation by living cells takes place in two steps – the initial rapid non-specific adsorption bythe cell wall and later slow active transport of metal ions into the cytoplasm (Mc-Eldowney, 2000). Heavy metal ions could be adsorbed by bacterial capsules, predominantly by carboxyl groups of polysaccharides. Extracellular biopolymers of*Enterobacterchloaceae*, *Marinobacter*species, *Klebsiellaaerogenes*, *Acinetobacter*species have been shownto accumulate metal ions (Scott and Palmer, 1990; Pirog and Пирог, 1999; Iyer*et al*., 2005; Bhaskar and Bhosle, 2006). *Pseudomonasaeruginosa*biofilm cells demonstrated considerably higher resistance to ions of copper,lead and zinc than planktonic (free-swimming) cells, while cells located at the periphery of the biofilm were killed (Ianieva, 2009). Extracellular polymers of the biofilm accumulated metal ions protecting bacterial cells inside the biofilm (Teitzel and Parsek, 2003).Kazy *etal.*(2002),studied exopolysaccharide (EPS) synthesis by copper-tolerant and copper-sensitive *P*. *aeruginosa* strains. Coper-tolerant strain produced twice as much EPS as sensitive strain. EPS production and copper accumulation in tolerant *P. aeruginosa* strain was induced by copper ions.

However,the inhibitory effectof metal ionson the synthesis of bacterial EPS was also observed. Richau *et al.*,(1997),obtained several mutants of*Sphingomonaspaucimobilis* tolerant to copper and defectivein synthesisof EPS gellan. As EPS synthesisis highly energy-consuming process authors explain the increase in copper tolerance of such mutants bythe decreased growth rate and the useofthe saved energy for protection against metal stress. The changes in plasma membrane permeability could prevent the entry of metal ions into the cell. *Escherichiacoli* mutants lacking porins-membrane proteins that act as channels for hydrophilic compounds exhibited low levels of silver ions accumulation inside the cell (Li *et al.*,1997).

2.4.2. Active transport of metal ions (efflux)

The largest group ofheavy metal resistance systems of bacteriais representedby active transport, or efflux. Bacteria exploit these systems to export metal ions from cells. Genetic determinants of efflux systems canbe localized on chromosomesand on plasmids (Nies, 2000). Some metal ions can enter the cell via the systems responsible for the uptake ofessential elements: for example, chromate is transported insidethe cell via sulphate transport system, ions of cadmium, zinc, cobalt, nickel and manganese enter the cells of *Ralstoniametallidurans* (*Alcaligeneseutrophus*) using systems of magnesium transport (Nies and Silver, 1989). ATP hydrolysisor electrochemical gradient (Rensing *et al.*,1999) are used to export metal ions from the cell. Efflux systems contain proteins belonging to three families: RND (resistance,nodulation,cell division), CDF (cation diffusion facilitator) and P-type ATPases. P-type ATPases and CDF proteins of gram-negative bacteria transport specific substrates through the plasma membrane into the periplasm. It should be noted that P-type ATPases predominantly transfer metal ions with high affinity for sulfhydryl groups

($Cu^{+/}Ag^{+}$, $Zn^{2+}/Cd^{2+}/Pb^{2+}$) while CDF-proteins specifically interact with ions of divalent metals (Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Fe^{2+}). Next transport complexes formed byRND proteins transport cations from the periplasm across the plasma membrane (Nies, 2003). Czc operon responsible for tolerance of the multiresistant bacterium,*R. metallidurans*CH34,tocopper, cobalt and zinc ions, encodes the best-studied efflux system utilizing energy of electrochemical gradient. $CzcCB_2A$ tfflux complex consists of 3 subunits CzcC, CzcB and CzcA. CzcA is RND-protein acting as a cation-proton antiporter; dimeric CzcB functions as the bridge between plasma and outer membrane. Despite the fact that CzcA protein may account for some level of heavy metal

resistance, CzcC and CzcB are essential for normal functioning of the efflux system (Nies, 2000).

The family of P-type ATPases includes transporters of mono- and divalent metal cations. CPx-type ATPases exporting monovalent copper and silver ions are found in*Enterococcushirae*(CopA and CopB) *Streptococcusmutans* and other bacteria (Odermatt *et al.*,1993; Vats and Lee, 2001). Some bacteria can employ other mechanisms of heavy metal resistance combined with efflux systems. For example, *P. putida* S4 strain transports copper ions by ATPase efflux system from the cytoplasm with subsequent sequestration in the periplasm. Another example of such dualtistic system is arsenic resistance ars system, composed of 3-5 genes and found bothin gram-positive and gram-negative bacteria. Ars operonencodes ATPasepump ArsA/ArsB and ArsC reductase.In the first step arsenate is enzymatically reduced to arsenite by cytoplasmic ArsC arsenate reductase and is exported by efflux system through the plasma membrane

2.4.3. Intracellular sequestration

Intracellular sequestration is the complexation of metal ions by various compounds in the cell cytoplasm. There are two classes of eukaryotic metal-binding peptides–metallothioneins and phytochelatins that are rich in cystein residues and bind metal ions by sulfhydryl groups (Pinto *et al.*,2003).Phytochelatins are low-molecular-weight peptides found in fungi and plants. They consist of 5-11 amino acid residues and are synthesized from glutathione(Clemens and Simm, 2003). Among prokaryotes the ability to synthesize metallothionein has been demonstrated for the Cyanobacterium,*Synechococcus*species,PCC 7942. This peptide contained fewer cysteine residues than the analogous eukaryotic peptide. Two genes smtA and smtB encode metallothionein synthesis by*Synechococcus*specieswhich is induced by cadmium and zinc ions

(Ybarra and Webb, 1999). Cadmium-tolerant *P. putida* strain possessed the ability of intracellular sequestration of copper, cadmium and zinc ions with the help of cysteine-rich low-molecular-weight proteins. Cellsof silver-tolerant *Pseudomonasdiminuta* strain produced several low and high-molecular-weight silver-binding proteins(Ibrahim *et al.*,2001). Some marine gamma-proteobacteria produced cadmium-inducible low-molecular-weight proteins similar to phytochelatins. Intracellular sequestration ofcadmium ions by gluthathione was observed in*Rhizobiumleguminosarum*cells (Lima *et al.*,2006).

2.4.4. Extracellular sequestration

Extracellular sequestrationistheaccumulation of metal ionsbycellular componentsinthe periplasm orthe outer membrane orcomplexation of metal ions as insoluble compounds. Copper-resistant *Pseudomonassyringae* strains synthesized copper-inducible proteins CopA, CopB (periplasmic proteins) and CopC (outer membrane protein) which bind copper ions and bacterial colonies turn blue as the result of metal accumulation (Cha and Cooksey, 1991). The similar blue bacterial colonies could be observed during growth of copper-tolerant *Pseudomonaspickettii*US321 strain on agar medium supplemented with copper, copper ions were accumulated in the periplasm or outer membrane. Authors suggested that the resistant strain accumulated copper as a complex and transportedit into cytoplasm while the sensitive strain accumulated copper in a free ionic form which is highly toxic for the cell (Gilotra and Srivastava, 1997). *Pseudomonasstutzeri*AG259 strain isolated from the soil of silver mine tolerated high concentrations of silver ions in the medium, silver resistance was plasmid-encoded. It was suggested that bacterium accumulated silver ions as sulphide complexes on the cell surface. Klaus *et al.*,(1999)demonstrated silver ion accumulation bycells of*P.*

*stutzeri*AG259 strain in the elemental form in the periplasm. Some bacteria would expel metal ions from the cytoplasm to sequester them within the periplasm.

Zinc ions exported from the cytoplasm by efflux system were accumulated in the periplasm of *Synechocystis* PCC 6803 strain, the similar strategy was employed by silver-tolerant *Salmonella*species strain and multi resistant *P. putida* S4 strain (Silver, 2003). Silver ions were specifically bound by the periplasmic protein SilEof*Salmonella*species strain and subsequently exported by ATPase pumps SilCBA and SilP. Another example of extracellular sequestration is metal precipitation as insoluble complexes. Sulfate-reducing bacteria generate large amounts of hydrogen sulfide that causes precipitation of a number of metal cations (Luptakova and Kusnierova, 2005; White and Knowles, 2000). *Klebsiellaplanticola* strain produced hydrogen sulfide from thiosulfate under anaerobic conditions and precipitated cadmium ions as insoluble sulfides, the same mechanism of cadmium precipitation was observed for *P. aeruginosa*strain under aerobic conditions. The formationof cadmium sulfide particles was detected on the surface of Cyanobacterium,*Nostocmuscorum*, heterotrophic bacteria associated with this cyanobacterium are implicated in their formation (Bekasova*et al.*,2000; Moskvina*et al.*,2003). Besides sulfides bacteria could precipitate metal ions as other insoluble compounds. *Vibrioharveyi* strain precipitated soluble divalent lead as complex lead phosphate salt.

2.4.5.Reduction of heavy metal ions by bacteria

Bacteria can reduce a broad spectrum of heavy metal ions (Table2.1). Bacteria reducing chromate, molybdate,and vanadate were isolated from various ecological niches(Smirnova, 2005). Some bacteria can use metals and metalloids as electron donors or acceptors for energy generation. Metals in the oxidized form could serve as terminal acceptors of electrons during

anaerobic respiration of bacteria. Enzymatical reduction of metal ions would also result in formation of less toxic form of mercury and chromium (Barkay *et al*., 2003; Viti *et al.,*2003).

Table 2.1: Reduction of metals and metalloids by bacteria

Reduction process	Microorganism
Hg^{2+}/Hg^{0}	*Bacillus cereus, Klebsiella pneumonia, P. stutzeri*
Fe^{3+}/Fe^{2+}	*Geobacter* species, *G. metallireducens, Bacillus thermoamylovorans*
Cr^{6+}/Cr^{3+}	*Desulfomicrobium norvegicum, Microbacterium* species, *Ochrobacterium intermedium, Brevibacterium* species, *Pseudomonas* species
As^{5+}/As^{3+}	*Staphylococcus aureus*
U^{6+}/U^{4+}	*Desulfovibrio desulfuricans, Shewanella putrefaciens, Thermoterrabacterium ferrireducens*
Mn^{4+}/Mn^{2+}	*Shewanella putrefaciens*
$Se^{6+}/Se^{4+}/Se^{0}$	*R. metallidurans*
Se^{4+}/Se^{0}	*Bacillus thermoamylovorans, Shewanella oneidensis*
V^{5+}/V^{4+}	*Shewanella oneidensis, G. metallireducens,*
Tc^{7+}/Tc^{4+}	*Geobacter sulfurreducens, S. putrefaciens*
Mo^{6+}/Mo^{5+}	*Thiobacillus ferrooxidans*
Au^{3+}/Au^{0}	*Strenotrophomonas* species
$Te^{4+}Te^{0}$	*Bacillus thermoamylovorans*

Source: Ianieva, (2009)

The best-studied system of metal detoxification by enzymatic reduction was one that confers tolerance to mercury encoded bymer-operon. Divalent mercury ions are transferred into the cell by MerT transport protein and are reduced to elemental mercury by MerA intracellular reductase (Brown *et al*., 2001).

2.4.6. Genetic determinants of heavy metal resistance of bacteria

Many bacteria are known to carry heavy metal resistant genes located either in their chromosomes or plasmids as shown in Table 2.2. The phenomenon ofthe plasmidor transposon localization of some genetic determinants of heavy metal resistance leadtothe conclusion that these genes could be transferred between bacteria by horizontal gene transfer (HGT) (Silver and Phung, 1996;Nies, 2003). HGT role in bacteria evolution under conditions of constantly changing environment orin the extreme habitats is confirmed by some ofthe following facts: frequent isolation of bacteria carrying plasmids and transposons from various environments; the phenomenon of natural transformation in bacteria; the occurrence of HGT in model ecosystems and in vivo; acquisition of new characteristics by autochthonous microbiota after introduction of plasmid-carrying bacteria into the environment (Coombs and Barkay, 2005).

HGT was proved tobe involved in evolution of genetic determinants for antibiotic resistance and mercury tolerance. HGT was shown to play role in evolution of genes responsible for metal ion homeostasis in bacteria inhabiting the deep terrestrial subsurface. Introduction of plasmids carrying genes for heavy metal tolerance into natural ecosystems however, have yielded inconsistent results (Smets*et al.*,2003).As metal resistance is frequently encoded by plasmid-borne genes and gene transfer by plasmids occurs in the natural environments, continuous metal stress could result in the selection of microorganisms harbouring resistance genes. Such

selection could benon-spcific;example,certain stress agent could lead to the development of bacterial resistance to different stress factors as plasmids could carry clusters of genes for resistance to several toxic agents. A good example ofsuch phenomenon would be the positive correlation between heavy metal and antibiotic resistance inbacteria (Wireman*et al.*,1997).

The incidence of plasmid-carryingbacteria was higher in the contaminated sites than in the undisturbed environments (Malik*et al.*,2002). However, there are reports about the presence of heavy metal resistant microorganisms in the non-contaminated sites (De*et al.*,2003). Such findings confirm the emergence of the systems of heavy metal resistance long before the anthropogenic pollution of the environment (Silver and Phung, 1996). Genetic determinants for metal resistance were first discovered on the bacterial plamids (Summers and Silver, 1972). Chromosomes of various bacteria were found to contain metal resistance systems similar to those found on plasmids. Ars operons on chromosomes of*E. coli, P. aeruginosa* and *Bacillus subtilis* structurally are similar to plasmid genetic determinants ars. However genetic systems of metal resistance on plasmids and chromosomes could differin some parameters: as a rule, essential metal ion homeostasis genes are located on chromosome while toxic metal resistance genes are plasmid-borne (Bruins *et al.*,2000). The best-studied metal tolerance system mer operon conferring reduction of mercury cations to elemental form has similar structure in various groups of bacteria regardless of its chromosome or plasmid location. The key genes ofmer operon aremerA (reductase gene), merT (transport protein gene), merP (extracellular mercury-binding protein gene) and merR (regulatory protein gene), however the operon may contain additional genes: *merB* (organomercurial lyase gene), *merC* (transport protein gene), *merD*(regulatory protein gene), merE (transport protein gene), *merF* (transport protein gene) and *merG* (gene conferring resistance to phenyl mercury) (Narita *et al*., 2003).

Table 2.2: Heavy metal resistance systems of bacteria

Genetic determinant	Location	Metal ion	Microorganism
czc operon	Plasmid PMOL30	Cd^{2+}, Zn^{2+}. Co^{2+}	*R. metallidurans* CH34
cnr operon	Plasmid PMOL28	Co^{2+}, Ni^{2+}, Cr^{6+}	*R. metallidurans* CH34
mer operon	Chromosome, Plasmid DU1358	Hg^{2+}	*B. cereus, Serratia marcescens*
ars operon	Plasmid773, Chromosome	AS^{5+}	*E. coli*
cadCA operon	Plasmid 1258	Cd^{2+}	*S. aureus*
czr operon	Chromosome	Cd^{2+}, Zn^{2+}	*P. aeruginosa*
Genes *cadA/cadR*	Chromosome	Cd^{2+}	*P. putida*
cop operon	Chromosome	Cu^{2+}	*E. coli*
sil operon	Plasmid pMG101	Ag^{+}	*Salmonella,* species

Source: Ianieva, (2009)

2.5. Chemical Surfactants

Surfactants (surface-active agents) are a diversegroup of chemicals consisting of a polar, water-solublehead group and a nonpolar hydrocarbon tail group, which is not as soluble in water (Ying, 2006). Surfactants are best known for their solubility and cleaning properties which secured them a place among detergents and other cleaning products. Massive quantities of surfactants are being used in households and industry every day, and most end up dispersed in different environmental compartments (soil, water, sediment). More than 4.2 million tonnes of detergent products and 1.2 million tonnes of softener products were used annually in Western Europe ten years ago (Peterssone*t al*.,2000).

In the same period the world production of synthetic surfactants was 7.2 million tonnes. In 2006, worldwide productionof surfactants rose to 12.5 million tonnes, and in 2007 over 3 million tonnes were produced in Western Europe alone. No doubt these figures will grow with ever growing detergent and cosmetics industry. After use, residual surfactants are discharged into sewage systems or directly into surface waters. They also accumulate in great quantities in wastewater treatment plants. Concentrations of surfactants or their degradation products vary in surface waters, sediments, and soils amended with sludge. For example, the concentrations/mass fractions of oneof the most common surfactants, linear alkylbenzene sulphonic acid (LAS), reached up to 1.1 mg L^{-1} in sewage effluents and up to 30.2 g kg^{--1} dry mass of treated sludge (Berna*et al*.,1989; Holt*et al*.,1998). Up to 0.4 mg L^{-1} of LAS was measured in surface waters (Fox*et al*.,2000). The elevated levels of surfactants in the environment can greatly affect theecosystem; their toxicity to organisms from mammals to bacteria is well known.

2.6. Chemistry of Surfactants

When dissolved in water at low concentrations, surfactant molecules exist as monomers. At higher concentrations, surfactant molecules aggregateinto micelles, reducing the system's free energy. The threshold concentration at which this occurs is known as the critical micelle concentration (CMC). Nonionic surfactants have lower CMC levels than anionic and cationic surfactants (Ying, 2006). Thisfundamental ability to form micelles gives surfactants their detergency and solubilisation properties. CMC also seems to define surfactant's antibacterial properties(Cella*et al*.,1955). Some of the commonly used surfactants are listed in Table 2.3, while Figure 2.1 shows their chemical structures. Surfactants are generally classified as anionic, cationic, amphoteric, and nonionic, depending on the charge of their head group.

Table 2.3: Names and abbreviations of the most common classes of surfactants

Common name	Abbreviation	Class
Linear alkylbenzene sulphonic acid	LAS	Anions
Sodium dodecyl sulphate	SDS	Anions
Alkyl sulphate	AS	Anions
Sodium lauryl sulphate	SLS	Anions
Alkyl ethoxysulphate	AES	Anions
Quaternary ammonium compound	QAC	Cations
Benzalkonium chloride	BAC	Cations
Cetylpyridinium bromide	CPB	Cations
Cetylpyridinium chloride	CPC	Cations
Hexadecyltrimethylammonium bromide	HDTMA	Cations
Amine oxide	AO	Amphoteric
Alkylphenol ethoxylate	APE	Nonionic
Alcohol ethoxylate	AE	Nonionic
Fatty acid ethoxylate	FAE	Nonionic

Source: Ivankovic and Hrenovic, (2010).

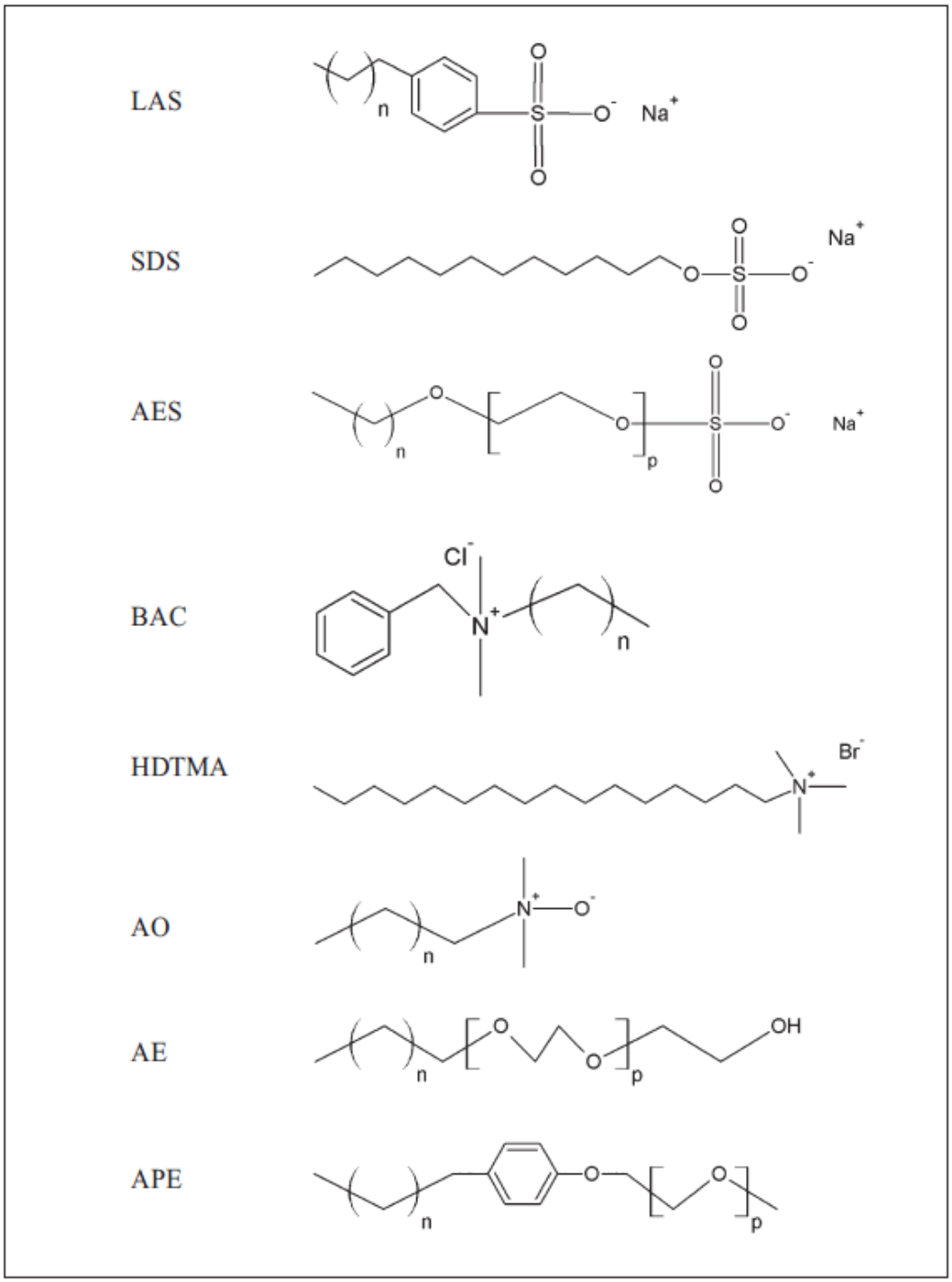


Figure 2.1. Chemical structure of some common surfactants
Source: Ivankovic and Hrenovic, (2010).

2.6.1. Anionicsurfactants

Anionics are historically the oldest and the most common type of surfactants; they are also the biggest in production and the largest species in various types of surfactants. When we think of detergents or common soaps, it is the anionic surfactants that do the washing. Anionic surfactants dissolve in water, generating negatively charged surface active group, whose aqueous solution is neutral or alkaline (Schmitt, 2001). The hydrophobic part of the molecule is usually an alkyl chain ofvarious length, alkylphenyl ether or alkylbenzene, and the hydrophilic part is carboxyl, sulphate, sulphonate, or phosphate. Except as detergents, they have successfully been in biotechnological and other industrial processes, including cosmetics industry (Cserhati*et al*., 2002). Anionic surfactants are also used in pharmaceuticalformulations to increase the efficiency of the active ingredients by direct binding to the drug (Seedher, 2000) or by enhancing adsorption or absorption and the partition of drugs between hydrophobic and hydrophilic compartments in organs and organisms. Some of the examples of anionic surfactants include linear alkylbenzene sulphonic acid, sodium dodecyl sulphate, alkyl sulphate, among others as could be seen in Table 2.3.

2.6.2. Contamination of waters by sodium dodecyl sulphate (SDS)

Sodium dodecyl sulphate (SDS) is an alkylsulphate with sodium as the counter ion with a chain length of 12 carbons (Cowan-Ellsberry *et al*., 2014). Sodium lauryl sulfate (SLS) and SDS are often used synonymously in reporting of product ingredients (Singer and Tjeerdema 1993). Concentrations >67% SLS (active ingredients) can be found in household products, dispersants, and herbicides (Lewis 1991; Singer and Tjeerdema 1993). Sodium dodecyl sulfate is not currently monitored in water systems or listed as a ground water contaminant (Kegley *et*

al.,2014). Other surfactants with similar uses are monitored (Singer and Tjeerdema 1993;Rebello *et al*.,2014).

In the United Kingdom, surfactant concentrations in surface waters have been recorded as high as 4161 g/L (Fox *et al*., 2000), while sewage effluents have had concentrations documented up to 1,090 lg/L (Holt *et al*.,1989). Treated sludge has been found to have concentrations of linear alkylbenzene sulfonate as high as 30,200,000µg/kg (dry weight) (Berna *et al*., 1989). All monitored concentrations for sulfates exceeded the predicted no-effect concentration value (250µg/L) for surfactants by Van de Plassche *et al*., (1999). In Massachusetts, the Town River had reported concentrations between 40µg/L and 590µg/L (Lewis and Wee, 1983), while other major rivers in the United States had reported surfactant concentrations that ranged from10µg/L to 3,300µg/L or 10µg/L to 40µg/L (Hennes and Rapaport, 1989).

2.6.3. Toxicity of sodium dodecyl sulphate (SDS) to different organisms

Sodium dodecyl sulfate was formally classified as 'environmental friendly' based on its readily biodegradable and low bioaccumulation properties, meaning it does not persist long in the environment (Belanger*et al*., 2009). Because of its fast acting, nonselective, and consistent toxicity, SDS is commonly used as a reference toxicant in toxicity tests (USEPA, 2002). Some studies have suggested that SDS can be lethal in acute exposures (Keller, 1993).In a study on anionic sodium dodecyl sulphate (SDS) toxicity to various taxa including different species of algae, crustaceans, echinoderms, and fish, the bacterium,*Vibrio fischeri*,proved to be the mostsensitive (Mariani *et al*., 2006). Tozum-Calgan and Atay-Guneyman (1994), reported that both growth and nitrogen fixation of the cyanobacterium,*Gloeocapsa,*were inhibited in the presence of SDS. Sewage sludge isolates,*Acinetobacterjohnsonii* and *Oligotropha carboxidovorans* showed 50 % and 20 % viability during treatment with 0.2 mg L^{-1} and 2 mg

L^{-1} of SDS, respectively (Malik *et al*.,2005).Chaturvedi and Tiwan (2013),also observed that anions surfactants (Sodium dodecylsulphate) are toxic for aquatic species like alage,crustetacean, echinodermata and fishes.

In a study on the toxicity of sodium dodecyl sulfate to federally threatened and petitioned freshwater mollusk species, it was shown that freshwater gastropods were more sensitive to SDS than freshwater unionids (Gibson*et al*.,2016).Developmental abnormalities in *Illyanassaobsoleta* embryos, such as incomplete or inhibited formation of lobe-dependent structures (e.g., foot, operculum, and eyes) of gastropods have been attributed to SDS exposure (treatments ranging from 10,000 – 30,000 µg/L) (Render, 1990). Tarazona and Nunez (1987), reported that SDS exposure significantly decreased shell weights in lymnaeid gastropods and impeded normal shell deposition (*EC50* = 540 µg/L for *Lymnaeavulgaris* and 610 µg/L for *Physaheterostropha*). When exposed to SDS (*EC50* = 31,400 µg/ L), Corbicula fluminea displayed avoidance behaviors and gill damage which decreased oxygen consumption and reduced siphoning activity (Graney and Giesy, 1988).

Sodium dodecyl sulphate has been reported to be toxic to aquatic plants. According to Dirilngen and Ince (1995), the toxic effects of SDS on the duckweed,*Lemna minor,*depended on the concentration; at lower concentrations, SDS increased its growth rate and inhibited it markedly at higher concentrations. Anionic SDS was equally observed to show toxic effects on juvenile sea bass (*Dicentrarchus labrax*), with mean *EC50*of 7.34 mg L^{-1}. The sensitivity of the sea bass was comparable to that of *Tigriopus fulvus* nauplii, and was lower than in marine bacteria, microalgae, or sea urchin (Mariani*et al*., 2006).

2.6.4. Toxicity of surfactants and metal ions on living organisms

Aquatic organisms are commonly exposed to several toxicants simultaneously in waters polluted by industrial, municipal or agricultural effluents. The toxicity of these mixtures may involve interactions between components which differ from the toxicities of the single components. Both heavy metals and surfactants are common aquatic pollutants and some investigations have shown that surfactants change the toxicity of metals to fish and to other aquatic organisms(Karbe, 1975; Swedmark *et al*., 1978). According to Seifert and Domka (2005), in concentrations higher than the threshold of toxicity, the surfactants and heavy metal ions studied lead to inhibition of the process of self-purification of ground waters and soil, and delay life-sustaining processes in the environment. Surfactants and heavy metal ions have also been reported to inhibit denitrification process by *Bacilluslicheniformis* by Seifert and Domka (2005).

2.7. Mathematical Models for Assessing Toxicity of Chemical Mixtures

Environmental exposures generally involve chemical mixtures instead of single chemicals (Moser *et al.*, 2005). Surveys of agricultural and urban streams and groundwater have brought public attention to wide spread chemical mixture contamination (Battaglin *et al*.,2003). Chemical mixtures are classified as either simple or complex mixtures. According to Feron*et al*.(1998), a simple mixture consists of a relatively small number of chemicals (e.g. ten or less), the composition of which is qualitatively and quantitatively known, example, a cocktail of pesticides or a combination of medicines. A complex mixture comprises tens, hundreds or thousands of chemicals, the composition of which is qualitatively and quantitatively not fully known, such as, a workplace atmosphere or drinking water.

The infinite number of potential chemical combinations (in terms of both constituents and concentrations of constituents) limits the utility of standard toxicity testing methods for establishing hazard associated with chemical mixtures. Modeling approaches could augment the standard toxicity testing paradigm when evaluating hazards associated with exposure to chemical mixtures. Chemical constituents of a mixturecan elicit similar action, dissimilar action or interaction (Casseet *al*.,1998). Models of mixture toxicity have focused primarily on quantifying the "no-interaction" scenario; while case of interaction often appear as qualitative observations (Hertzberg and MacDonell, 2002).Concentration addition (Loewe additivity) and response addition (Bliss independence) (Greco *et al*., 1992) are commonly used to model the toxicity of non-interacting chemicals within a mixture.

2.7.1. Non-interaction mixture model

This comprises the concentration addition model and independent action model. In this zero interaction model, effects stronger than expected are often designated as resulting from synergism and effects smaller than expected can be designated as resulting from antagonism (Groten *et al*.,2001).

2.7.1.1.Concentration addition model

Concentration addition model (CA) relies upon the assumption that mixture componentscontribute to toxicity through a common mechanism of action or that they act on the same biological target and therefore could be viewed as being dilutions of each other, each having a different potency (Sorensen *et al*., 2010). Calculating mixture toxicity based on concentration addition requires assessing the relative contribution of each constituent to the total toxicant pool. The toxicity of this pool is then modeled as a single toxicant. Concentration addition is the basis of the "toxic equivalency" approach commonly used to assess toxicity of

chemicals of the same class such as dioxins (Safe, 1990). Ample evidence supports the use of the concentration addition model for assessing mixture toxicity of like-acting chemicals (Altenburger *et al*., 2000; Cedergreen, 2014;).

Mixture Modeling: The joint toxicity of binary mixtures of like-acting chemicals could be computed as described by Olmstead and LeBlanc, (2005).

$$R = \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{C_1}{EC_{50i}} \right)^p}}$$

Where R is the response to the mixture, C_i is the concentration of chemical i inthe mixture, EC_{50i} is the concentration of chemical i that causes a 50% response, and p is the average power associated with the chemicals in the cassette. The average power was used because chemicals within a cassetteshould have similar slopes.

2.7.1.2. Independent action model

The indepent action or response addition model has been used to compute toxicity of mixtures where chemical components have different mechanisms of action (Backhaus *et al.*, 2000; Walter *et al*.,2002). In this model, combined effects of the chemicals are based on the probability that individual constituents of the mixture will affect the exposed organisms. According to Olmstead and LeBlanc (2005), the concept of response addition was used to compute the joint toxicity associated withthe different chemical cassettes within a mixture. The response addition modelwas used because each cassette is assumed to elicit a response through differentmechanisms. The response addition model can be depicted as:

$$R = 1 - \prod_{i=1}^{n} (1 - R_i)$$

Where R represents the response to the mixture and R_i is the response tochemicals in cassette i.Equations 1 and 2 were integrated to establish the response associated withindividual cassettes within a mixture and to sum the responses associated withthe cassettes (Olmstead and LeBlanc, 2005). The resulting equation isa combination of concentration and response addition equations:

$$R = 1 - \prod_{i=1}^{n}\left[1 - \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{C_i}{EC_{50i}}\right)}}\right]$$

2.7.2. Mixture Interaction

The concentration addition and independent action models are limited in their application to complex mixtures in that they do not address chemical interactions. Toxicokinetic interactions can occur between chemicals in which one of the chemicals alters the effective concentration of another (Andersen and Dennison, 2004). Alternatively, toxicodynamic interactions can occur between chemicals in which one chemical influences the response of the organism to another chemical (Andersen and Dennison, 2004). Both toxicokinetic and toxicokinetic interactions can significantly impact the toxicity of chemical mixtures.

2.7.2.1 Chemical interactions model

The ability of one chemical in the mixture tomodify the effective concentration of another was defined by coefficients ofinteractions or K-functions (Finney, 1942; Mu and LeBlanc, 2004). According to Rider and LeBlanc (2005), specifically,K-functions, defined the degree to which the concentration of PBO in themixture altered the effective concentration (i.e. oxon metabolite) of organophosphatein the mixture. K-functions were described by

experimentallyderiving the effect of concentrations of PBO on the *EC50* values derived foreach organophosphate. K-functions were calculated for each of the PBO concentrations with the following equation:

$$K = \frac{EC_{50OP}}{EC_{50OP+PBOx}}$$

Where EC_{50OP} is the concentration of organophosphate that immobilized 50%of the exposed animals and $EC_{50OP+PBOx}$ is the EC_{50} of the organophosphatewhen exposure occurred in the presence of x concentration of PBO. These K- functionswere then plotted against the concentration of PBO from which theywere derived. The logistic equation that defined this relationship was used tocalculate K-functions when modeling mixture toxicity. K-functions wereintegrated into this model to describe toxicokinetic interactions between PBOand the organophosphates:

$$R = 1 - \prod_{i=1}^{n} \left[1 - \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n} \frac{ka.i\,(c_a)\,xc_i}{EC50_I} \right)^p}} \right]$$

Where $k_{a,i}$ represents a function describing the extent to which chemicala (PBO) present in the mixture at concentration C_a alters the effectiveconcentration of chemical i (malathion or parathion) (Rider and LeBlanc 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area

Owerri lies within latitude 05° 29´ 06S and longitude 07° 02´ 06S in southeastern Nigeria. It is situated at an elevation of 73 meters above sea level. The city experiences a wet season from April to November and a dry season for the rest of the year (Victor *et al*.,2011). Mean daily maximum temperature is between 28 and 35 °C, while daily minimum values range between 19 and 24 °C. The Otamiri River is one of the two major surface waters that traverse the city. This river runs south from Egbu where it has its major base past Owerri and through Nekede, Ihiagwa, Eziobodo, Olokwu Umuisi, Mgbirichi, Umuagwo and finally to Ozuzu in Etche town of River State of Nigeria, from where it finally joins the Atlantic Ocean. The length of the river from its source to its confluence at Emeabiam with the Uramiriukwa river is 30 kilometers. The Otamiri watershed covers about 10,000 square kilometers with annual rainfall of 2,250 to 2,500 millimeters. The water shed is mostly covered by depleated rain forest vegetations (Onweremadu *et al*., 2008). The location map of the study sites is shown in Figure 3.1.

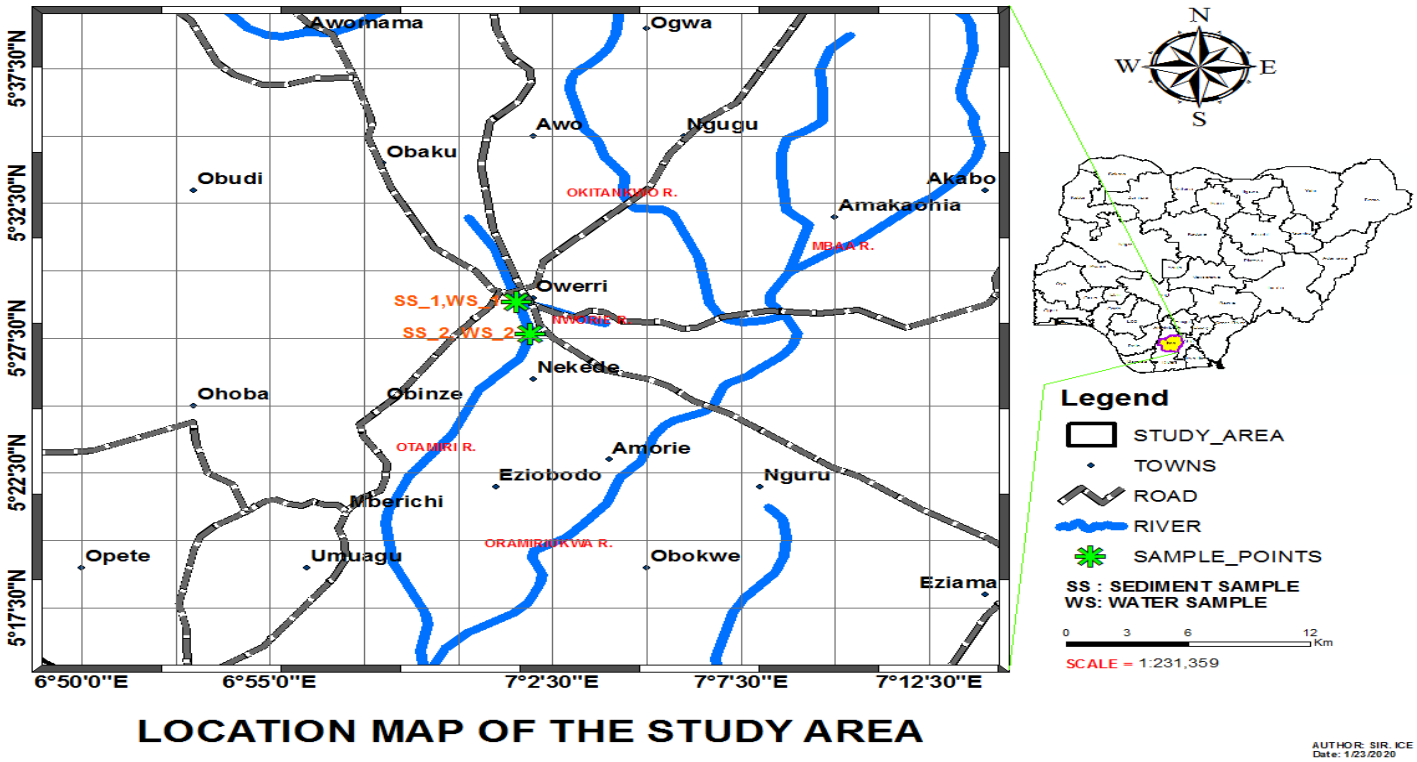


Figure 3.1: Location Map of the Study Area, Showing Sampled Points

3.2. Samples Collection

Water and sediment samples were collected along the course of the river,adjacent to motor mechanic village, Nekede. Two sampling locations were used for the study. Thefirstlocation was approximately 100 meters downstream from the point Nworie river joined with Otamiri River (5.465^{o}N, 7.035^{o}E), while the second location was also 100 meters from the first location (5.463^{o}N, 7.034^{o}E). These sampled points are shown in green colour in Figure 3.1. The samples were collected as described by Nweke *et al*.,(2007a). Eckman grab sampler was used for the collection of the sediment samples at the two locations, which were later pooled together to form composite sample in clean cellophane bag. The water samples were collected with four750-ml sterile plastic containers(two for each sampling point)and were also pooled together in two 1.5liters'sterile plastic containers.These containers were previously sterilized by soaking for 30 minutes in 70% ethanol and rinsed severally with sterile water.All the samples were stored in coolers and taken to the laboratories for analysis. The water samples were stored in the refrigerator at 4^{o}C, until required.

3.3.Physicochemical Analysis of the Water Sample

3.3.1 Determination of pH

The pH was determined using a digital Labtech pH meter, Jenway (Model type HANNA 1910). The pH meter was calibrated using standard buffers of pH 4, pH 7, pH 10. The electrode of the standard buffer in turn was rinsed with distilled water, cleaned with a soft lint-free tissue before dipping into the next buffer. The temperature compensator was set at the appropriate temperature before the calibration. After the calibration, the electrode was rinsed and cleaned. The pH of the sample was measured by dipping the electrode which was rinsed with distilled

water for usage on subsequent samples. The temperature knob was set at the temperature of each of the samples and the pH reading was obtained from the digital readout (AOAC,1990).

3.3.2 Determination of temperature

The temperature of the water sample was determined using mercury in glass thermometer. The measurement was done after the sample was collected and the thermometer was immersed into the water sample and read. The thermometer used was calibrated in degree Celsius, having standard range of 0-100°C (AOAC, 1990).

3.3.3 Determination of conductivity

The conductivity measurement was carried out using HANNA EC 215 conductivity meter. The conductivity meter was calibrated with a standard 0.01MKCl solution having a conductivity of 1413uS/cm.The conductivity cell was rinsed with a soft lint-free tissue. The conductivity of the sample was determined by dipping the cell into the sample and rinsing the cell with deionized water. The conductivity reading was obtained from the digital read out (AOAC,1990).

3.3.4 Determination of total hardness

Twenty-five millilitres (25ml) of the water sample was added into a 100ml conical flask, containing 1ml of ammonia/ammonium chloride. A 2-ml portion of buffer of pH10 was added to the water sample and thereafter, 3 drops of Erichrome Black-T indicator was added to the sample.The solution was titrated with 0.01M EDTA solution using a microburette and colour change from wine red to blue indicates the end point. Conductivity was determined using EDTA titrimetric method and methyl orange method respectively as described by APHA, (1998).

$$\text{Total hardness (Mg/l } CaCO_3) = \frac{\text{Vol. EDTA (titre) x m(EDTA) x100 x 1000}}{\text{Vol. of Sample}}$$

3.3.5 Determination of chloride

Twenty-five millilitres (25ml) of the water was pipetted into a 100ml conical flask. A portion of 1ml of potassium chromate indicator was added to the water sample. The solution in the conical flask was titrated with 0.02 ml silver nitrate to a reddish brown end point using a microburette.A blank titration was done as above using deionized water. Chloride was determined by estimating in accordance to Argentometric method described by APHA,(1998).

$$\text{Chloride (Mg/l)} = \frac{(\text{Sample titre} - \text{Blanc titre}) \text{ x } 0.02\text{m} \text{ x } 35.5 \text{ x } 1000}{\text{Vol. of Sample}}$$

3.3.6 Determination of turbidity

Turbidity was determined by photometric method described HACH spectrophotometer at wavelength of 860nm and programme number 750. Twenty-five millilitres (25ml) of filtered deionized water was measured into a 25ml sample cell bottle as blank. The blank was used to zero the spectrophotometer. Then the sample was vigorously shaken and 25ml of the sample was put into the light shield and closed after the blank was removed and the read bottom was pressed. The value was then displayed in mg/l (AOAC,1990).

3.3.7 Determination of phosphate

In determination of phosphate according to US-EPA, (2000), 6.0g ammonium heptamolybdate was weighed and dissolved in 150ml distilled water in 250ml conical flask. 2.6g of ascorbic acid was dissolved in 50ml of distilled water in 1 litre volumetric flask to give 0.0007M. Then 0.4g of potassium antimony tartrate was also weighed and dissolved in 20ml distilled water (0.0000086M). 0.1M stock of concentrated sulfuric acid was prepared by dissolving 10ml of the stock in 50ml distilled water.

3.3.7.1 Preparation of phosphate stock and working standard

One-thousand miligramsper litre (1000 mg/L) PO_5 was prepared by weighing accurately 1,532mg of potassium phosphate trihydrate in 250ml of distilled water. 0.5, 1.5, 2.0 and 2.5ppm PO_5 were prepared by proper dilution with distilled water for calibration curve. Thereafter, 12.4ml of the ammonium molybdate solution was transferred into a 50ml volumetric flask and then 10ml sulphuric acid was added and swirled, then followed by 2.3ml of antimony potassium tartrate. The mixture was swirled properly to mix and the mixture was made up to the mark with distilled water. Spectrophotometeric determination was carried out by adding 0.4ml molybdate reagent to 20ml of standard or sample in a test tube and swirled to mix. Also, 0.4ml of L-Ascorbic acid was added and swirled. The light absorption of the solution was measured at 820nm wavelength (AOAC, 1990).

3.3.8 Determination of biological oxygen demand (BOD)

The BOD_5 was determined using DO_2 meter. The DO_2 meter was calibrated using 5% Sodium sulphate solution. The probe of the meter was then inserted into the sample after the meter was switched on for about 10minutes. The reading was recorded in mg/l. The sample was then incubated in a 250ml Winkler's bottle for a period of 5days at 20°C.Then the DO_2 at the fifth day was recorded by inserting the probe again into the sample. The difference between the DO_2 (1) and DO_2 (2) was recorded as the BOD_5(APHA, 1998).

$BOD_5 = DO_2(_1) - DO_2 (_2)$

Alternatively,

$BOD_5(mg/l) = F (T_0 - T_5) - (F-1) (D_0 - D_5)$

Where:

D_0=Average O_2 content of dilution water at the beginning of the assay(mg/l).

D_5=Average O_2 content of dilution water after 5days incubation

T_0=O_2 content of one of the sample dilution at the beginning

T_5=O_2 content of one of the sample dilution after 5-day incubation.

F = Dilution factor such that $0.40<T_0-T_5<0.6T_0$

3.3.9 Determination of dissolve oxygen (DO)

The DO_2 (Dissolved Oxygen) was determined using EXTECH Model DO 700 digital Dissolved Oxygen meter. The DO_2 meter was calibrated using 5% Sodium sulphate solution. The probe of the meter was then inserted into the sample after the meter was switched on for about 10minutes. The reading was recorded in mg/L (APHA, 1998).

3.4. Physiochemical Analyses of Sediment Sample

3.4.1. Sample preparation

The sediment sample was taken to the laboratory and air dried, clumps broken or crushed with porcelain mortar and sieved with 2mm stainless metallic sieve (Fawole and Oso, 2004).

3.4.2. Determination of Sediment pH

To ten grams (10.0g) of the air-dried sediment sample in a 100-ml beaker, was added10ml of distilled water and the suspension formed allowed to stand for 15minutes, with frequent stirring with a glass rod. Prior to usage, the pH meter was calibrated with pH 4.0 and 7.0 buffers. The electrode of the pH meter was then immersed into the partly settled suspension and the pH determined. The procedure above was repeated using 0.001M Calcium chloride solution (prepared by dissolving 1.1g of Calcium chloride salt in 1000ml of distilled water) and the pH was then measured (AOAC,1990).

3.5. Methods for Heavy Metal Analysis

Heavy metal analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA, (1998).

3.5.1. Sample digestion (sediment)

This was carried out according to Adrian, (1973). Approximately two grams (2 g) of dried sediment sample were weighedinto a digestion flask and 20ml of acid mixtures (650ml concentrated HNO_3; 80ml perchloric acid; 20ml concentrated H_2SO_4) was added,thereafter, the flask was heated until a clear digest was obtained. The digest was diluted to 100 ml mark, with distilled water.

3.5.2. Sample digestion (Water)

Procedure: The sample was thoroughly mixed by shaking, and 100ml of it was transferred into a 250 ml glass beaker, to which 5.0ml of concentrated nitric acid was added and heated to boil till the volume was reduced to about 15-20ml, by adding 5ml increments ofconcentrated nitric acid till all the residue was completely dissolved. The mixture was cooled, transferred into a 100 ml volumetric flask and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed (Adrian, 1973).

3.5.3. Preparation of reference solutions

A series of standard metal solutions in the optimum concentration range were prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5ml of concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations (Adrian, 1973).

3.6. Determination of Anionic Surfactants

3.6.1 Preparation of samples

This was done according to AOAC, (1990).

3.6.1.1. Soxhlet extraction method

Ten grams (10g) of the homogenized sample was mixed with 20g of anhydrous sodium sulphate in agitate mortar to absorb moisture. The homogenate was then placed in extraction cellulose thimble (33.94mm), covered with a Whatman filter paper and inserted into a soxhlet extraction chamber of the soxhlet unit. Extraction was carried out with 200ml ethanol or ethylacetate for 3hours. The crude extract obtained was evaporated using a rotary vacuum evaporator at 40^{o}C, just to dryness (AOAC,1990).

Florisil Clean up: Florisil was heated in an oven at 130^{o}C over night (Ca. 15h) and transferred to a 250ml beaker and placed in a desiccator. A 0.5g anhydrous Na_2SO_4 was added to 1.0g of activated florisil (Magnesium silicate)(60-100nm mesh) on an 8ml column plugged with glass wool. The packed column was filled with 5ml of n-hexane for conditioning. The stopcock was opened to allow n- hexane run out until it just reaches top of sodium sulphate into a receiving vessel whilst tapping gently the top of the column till the florisil settled well in the column. The extract was transferred on to the column with disposable Pasteur pipette from an evaporating flask. Each evaporating flask was rinsed twice with 1ml portion of n-hexane and was added to the column. The eluate was collected into an evaporating flask and rotary evaporated to dryness. The dry eluate wasdissolved in 1ml n-hexane for analysis using Gas Chromatography. For the river water sample, a portion of the water sample was mixed with equal volume of n-hexane and shaken very well before transferring to separating funnel, where it forms two layers. The water layer was removed, while the n-hexane layer was collected, concentrated by

evaporation and then analysed, using Gas Chromatography by evaporation and then analysed, using Gas Chromatography (AOAC,1990).

Fixed Setting: generally, gas flows to the columns, the inlets, detectors, and split ratios was adjusted. In addition, the injector and detector temperatures were set. The detectors were usually held at the high end of the oven temperature range to minimize precipitation. Ordinarily, all these parameters should have been set to the correct values, but were double checked: Buck 530 gas chromatography equipped with an on-column, automatic injector, flame ionization detector, HP88 capillary column (100m x 0.25µm film thickness), CA, USA.

Detector temperature A: 250^{o}C

Injection temperature 22^{o}C

Integrator chart speed: 2cm/min

Set the OVEN TEMP at 180^{o}C and allow the Gas Chromatographic machine to warm, while its warming set:

Temperature Condition

Initial Temp	**Hold**	**Ramp**	**Final Temp**
60^{o}C	5min	10min	200^{o}C
200^{o}C	2min	5min	300^{o}C

When the instrument is ready, the "NOT READY" light will turn off and then begin your run. Inject a 1.0 µL sample onto column A, using proper injection technique (AOAC,1990).

3.7. Bacteriological Analysis of the Samples

One gram (1 g) of the sediment sample was suspended in 9 ml of distilled water contained in 100 ml flask and shaken vigorously for 60 seconds and allowed to stand for about 10 minutes as described by Fawole and Oso (2004). Ten-fold serial dilutions of sediment suspension and water samples were carried out as described by Fawole and Oso (2004). One milliliter (1ml) of the sediment suspension or water sample was aseptically collected using sterile Pasteur pipette and placed in nine milliliters (9ml) of distilled water in a test tube and shaken gently for 60 seconds. From this tube, one milliliter (1ml) of the dilution was transferred to the next tube containing nine milliliters of distilled water. This dilution was continued to the sixth tube, after which, one milliliter of the dilution was discarded from the last tube. Then 0.1ml of the 10^{-5} dilutionof the sediment suspension and10^{-3}dilutionof the water samples were aseptically inoculated onto sterile Nutrient agar plates in triplicates, using sterile Pasteur pipette and then spread with sterile glass rod and incubated at 37^{0}C for 24 hours. The bacterial colonies were counted to determine the total heterotrophic counts (CFU/mL) of the samples. Plates that had 30-300 colonies per plate were selected for counting. The total heterotrophic counts were determined by dividing the average number of colonies per plate by the sample volume (0.1 ml). Discrete colonies were further subcultured on Nutrient agar plates to obtain pure cultures, which were then stored on agar slants in the refrigerator at 4^{o}C. The isolates obtained were identified using morphological characteristics, Gram staining, spore staining and biochemical tests (Cheesbrough, 2005). The percentage occurrence of each isolate in each sample was determined as follow**:**

$$\text{Occurence } (\%) = \frac{\text{Number of colonies of isolate A}}{\text{Total number of isolates}} \times 100 \qquad (14)$$

3.7.1. Gram staining techniques

A smear of each of the bacterial isolates was made on a clean grease-free glass slide and fixed by air drying and passing through the bursen flame three times. The smears were then covered with 0.3% Crystal Violet stain for 60 seconds and rapidly washed off with distilled water thereafter. The smears were then covered with Lugol's iodine for 60 seconds and washed off with distilled water. The smears were decolorized with a mixture of acetone-alcohol and washed off after 10 seconds, with distilled water. The smears were finally flooded with three drops of 0.1% Safranin for 2minutes and washed off with distilled water. The back of the slides was then wiped and placed in a draining rack for the smear to dry before they were viewed under the microscope, with a drop of oil immersion and x 100 objective lens (Cheesbrough, 2005). Gram positive bacteria gave purple coloration while gram negative bacteria gave pinkish coloration.

3.7.2. Spore staining

A smear of each of the bacterial isolates was made on clean grease–free glass slide and fixed by air drying. The smears were then covered with malachite green stain and placed over steam for 5 minutes while topping the slides with more malachite green stain, when it dries out. At the end of 5 minutes, the stain was rinsed off with clean water and counter stained with Safranin for 2 minutes and washed off with water. The smears were then allowed to dry before they were viewed under the microscope, with x 100 oil immersion objective lens, with a drop of immersion oil (Cheesbrough, 2005). Spore positive slides gave green color while negative slides gave only pinkish coloration.

3.7.3. Motility test

This test differentiates bacteria that are motile from those that are non motile based on the presence or absence of flagella respectively. The test was carried out using Sulphide Indole Motility (SIM) agar. A loopfull of each of the test organism (culture) was aseptically inoculated into SIM agar using stab inoculation technique. The tubes were marked and were incubated at 37^0C for 24hours. At the end of the incubation period, the tubes were checked for growth by the level of extension of the growth in the incubated tubes. Extension of the growth from the inoculated position indicates positive test while none-extension is an indication of a negative test (Chessbrough, 2005).

3.7.4. Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase such as *Staphylococcus* from non catalase producing bacteria. Two millitre (2ml) of hydrogen peroxide solution was poured into several test tubes for each of the bacterial culture. Using a sterile wooden stick, each colony of the bacterial isolates was immersed in each of the hydrogen peroxide solution. Active bubbling within 10 seconds was an indication of a positive test while none was an indication of a negative test (Cheesbrough, 2005).

3.7.5. Citrate utilization test

This test helps in the identification of Enterobacteriaceae. A loopful of culture of each of the test organisms was inoculated onto sterile agar slopes of Simmon citrate agar, using stab inoculation technique. The inoculated agar slopes were then incubated at 37^oC for 24 hours. A bright blue coloration was an indication of a positive test while none was an indication of a negative test (Cheesbrough, 2005).

3.7.6. Indole test

Some microorganisms are capable of hydrolyzing the amino acid Tryptophan and one of the end products is indole. The ability of a microbe to carry out this reaction can be used for biochemical characterization. The culture or colonies of test organism was suspended in sterile peptone (about 3ml) preparation in sterile test tubes and incubated at 37^{o}C for 24 hours after which 0.5ml of Kovac's reagent was added and shaken gently. A red coloration on the surface layer within 10 minutes was an indication of a positive test while none was an indication of a negative test (Chessbrough, 2005).

3.7.7.Sugar fermentation/Hydrogen sulphide production test

Each colony of the different test organisms was inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated agar slopes were incubated at 37^{O}C for 24 hours. The different colors of the slopes and butts in addition to the presence of gas production and hydrogen sulphide (H_2S) blackening was indicative of the type of bacteria present (Chessbrough, 2005).

3.7.8. Molecular identification of the preponderant isolates

The isolates with the highest percentage occurrence from the river water and sediment samples were selected for the toxicity assay. The identities of these preponderant bacteria were further confirmed through molecular analyses, using 16S rRNA gene partial sequencing.

3.7.8.1.DNA extraction

The genomicDNAwasextractedusingQuick-DNA^{TM}miniprepplus kit(ZymoResearch),accordingto the recommendedprotocol.

Protocol

Agarose gel electrophoresis

A 2%agarosegelwaspreparedbydissolving 1.2gofagarosein 60mlof1XTAEbuffer. Themixturewasheatedtoa clearsolutionusingamicrowaveovenandallowedtocooltoabout 50 ºC.3*µl*ofethidiumbromidewasaddedintothesolutionandmixedthoroughly.Theagarose preparationwascarefullypouredintoageltray,withthegelcombinplaceandallowedto solidify.Thetraywasloadedintothegeltankand1XTAEbufferwaspouredintothetank,making surethatthegelwasproperlysubmerged.Thegelcombwascarefullyremoved.5*µl*ofDNAwas mixedwith2*µl*ofloadingdyeandloadedintotheholes.Thetankwasconnectedtothepower packand settorunat100voltsfor20minutes. Thebandwereviewedusing thegel documentationsystem.

3.7.8.2.PCR protocol

12.50 µl of one *Taq* quick-load 2x master mix with standard buffer (New England Biolabs Inc.), 0.3 µl each of forward and reverse primers, 10.9 µl of nuclease free water and 1 µl of DNA template was used to prepare 25µl reaction volume of the PCR cocktail. The reaction was gently mixed and transferred to a preheated thermocycler.

Amplification conditions for the PCR were as follow. 3 minutes at 94^{o}C to denature the DNA, followed by 30 cycles of denaturation at 94^{o}C for 30 seconds, primer annealing at 56^{o}C for 45 seconds and strand extension at 68^{o}C for 5 minutes on an Eppendorf Nexus Gradient Master cycler (Germany), PCR products were seperated on a 2% agarose gel and DNA bands were visualised with ethidium bromide.

3.7.8.3.Sequencing protocol

PCR products were cleaned using Exo/SAP protocol prepared by adding exonuclease I (No. NEBM 0293L) 20 U/ul and shrimp alkaline phosphatase (No. NEBM 0371) 1U/µl, and amplified PCR product, to a 0.6 ml mocro-centrifuge tube. These were mxed well and incubated at 37^{o}C for 15 minutes. The reaction was stopped by heating the mixture at 80^{o}C for 15 minutes.

Fragments were sequenced using the Nimagen, Brilliant Die ^{TM} Terminator code sequencing kit V3.1, BRD3-100/1000 according to the manufacturer'sinstructions.The labelled products were then cleaned with the ZR-96 DNA sequencing clean-up kit catalogue No. D4053). The cleaned products were injected on the applied biosystems ABI 3500XL Genetic analyser with a 50 cm array, using POP7. Sequence chromatogram analysis is performed using FinchTV analysis software.

3.8. Dehydrogenase Activity Assay

3.8.1. Test organisms

The test bacteria were those that recorded the highest percentage occurrence in Otamiri river water and sediment respectively,and were subsequently adopted for the toxicity assay.

3.8.2. Culturing of test bacteria for toxicity assay

Nutrient Broth was prepared according to the manufacturer's instruction. 1.3g of the medium was weighed into a 250-ml conical flask, and then 100ml sterile deionized water was added and shaken to dissolve. Fifty millilitres (50ml) of the broth were dispensed into each of the two 100-ml conical flask; the flasks were capped and sterilized in an autoclave at 121^{o}C, 15psi for 15 minutes. When cool, the flasks were aseptically inoculated with the test bacteria and thereafter, placed on a rotary incubator (150 rpm) at room temperature (28 ± 2°C) for 16 to 24 hours (Nweke*et al*., 2014).

3.8.3. Harvesting and washing of bacterial cell

These were carried out as described byNweke *et al*. (2014). The cells were harvested by centrifugation at 3000 rpm (Newlife Centrifuge, NL80-2) for 15 minutes. Harvested cells were washed twice in sterile deionized water to avoid any nutrient carryover. Washed cells were re-suspended therein and the cell density adjusted to 0.1 at 540 nmwavelength in a Spectrophotometer**(**VIS Spectrophotometer 721D**).**This 0.1 cell density cell suspension was equivalent to $1.1x10^8$cells/ml based on Mc-Farland standard. The cell suspensions were used as inocula in the toxicity assay.

3.8.4.Preparation of metals and SDS stocks

The Ni(II), Cd(II), Co(II), Pb(II) and Zn(II) ions were used as $NiSO_4.6H_2O$, $CdSO_4.8H_2O$, $ZnNO_3.6H_2O$, $Pb(NO_3)_2$, and $CoCl_2$.All the reagents (Appendix VI)were of analytical grade and were sourced from Sigma-Aldrich (Germany). Stock solutions of 10 and 50mM were prepared in sterile deionized water for the individual metal ions and SDS respectively. The metal solutions (10mM) were prepared in separate 100-ml volumetric flasks by dissolving 0.263g of Ni(II), 0.257g of Cd(II), 0.298g of Zn(II), 0.331g of Pb(II) and 0.130g of Co(II),in 20ml sterile deionized water and made up to 100ml. Fifty millimolar (50mM) solution of SDS was prepared by dissolving 1.442g of SDS, in 20ml of sterile deionized water and made up to 100ml in a 100-ml volumetric flask. All reagent solutions were sterilized by membrane filtration (Sartorius membrane filter with pore size 0.45µm).

3.8.5. Preparation of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-Tetrazolium Bromide (MTT-Indicator stock)

The 0.1% solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) stock was prepared by dissolving 0.1g of MTTin 20ml of sterile deionized water and made up to 100ml,thereafter sterilized by membrane filtration and stored in 100-mlconical flask wrapped in aluminum foil for use. Trial runs were carried out with different concentration range of the individual toxicants (SDS and metal ions) against 0.1% MTT-indicator as shown in Appendix VI.

3.8.6. Design of experimental protocols

The volume of MTT andbacterial culture were constant at100µl (0.1ml) each, while that of the nutrient broth was constant at 500µl (0.5ml). Different protocols were generated for the individual toxicants (heavy metals and SDS), as well as the various mixture combinations (AppendicesVIIa-VIIzii.).Protocols were prepared with toxicantsworking concentration ranges of 0-1 mM and 0-10 mM for heavy metals and SDS respectively, using the dilution formular as shown in Appendix V.

3.8.7.Fixed ratio design

The dehydrogenase activity assay wascarried out using fixed ratio experimental design (Nweke*et al*., 2016; Nwanyanwu*et al*., 2017).In each mixture, the mixture ratio was kept constant, while the total concentration of the mixture was varied to obtain a complete dose-response relationship of the mixtures. The response or end point was the inhibition of dehydrogenase activity in the test bacteria.

3.8.7.1. Design of SDS and individual metal ion experiments

The reaction mixture consisted of 2-ml final volumes of low-strength nutrient broth, supplemented with varying concentrations of SDS or metal ions. Into each 15-ml screw capped culturetube containing 0.5 ml portion of x4-strength nutrient broth (pH 7.0), requisite volumes of sterile deionized water and stock solutions of respective metal ionor SDS were added.

The final amount of nutrient broth in the reaction mixture was 0.2% w/v. Thereafter, 0.1ml of 0.1% aqueous solution of MTT and 0.1ml of the standardized bacterial suspension were added into each tube to obtain varying concentrations of metal ionor SDS. Each concentration of SDS as well as the individual metalion was prepared in duplicates. Controls were prepared without the toxicants. Duplicate control tubes were prepared for SDS and each metal ion, giving a total of 12 controls. The cultures were incubated at room temperature ($28 \pm 2^{o}C$) for 24 hours.

3.8.7.2.Design of SDS and metal(s) mixture ratios

The binary, ternary, quaternary, quinary and senary mixtures of SDS with the five heavy metals(Ni, Cd, Pb, Zn and Co) were studied using fixed ratio design as a function of weight to weight ratios. The50% equi-effect concentration ratio (EECR 50) as determined from the $EC_{50}$of the individual mixture components, as well as three arbitrarily choosen concentration ratios (ABCR) were designed for the bioassays, as shown in Tables 3.1 to 3.4 below. For the binary mixtures, the mixtures were combined as p (%) = SDS and 100-p (%) = metal ions. The mixtures were prepared from the 10 mM and 50 mM stock concenteations (for heavy metals and SDS) by combining requisite volumes of the heavy metals and SDS stock solutions to produce a particular concentration ratio. For every mixture, at a constant mixture ratio, the total concentration of the components was varied to obtain the complete dose-response relationship. The mixtures were studied as a single toxicant solution during the toxicity assay.

Table 3.1: Equieffect Concentration Ratio (EECR50) and Arbitrary Concentration Ratio (ABCR) of Binary and Ternary Mixtures for *S. marcescens* (SerEW01).

Mixtures	**Mixture Ratio (%)**									
Metal+SDS	Ni(II)	SDS	Cd(II)	SDS	Pb(II)	SDS	Zn(II)	SDS	Co(II)	SDS
EECR50	6.51	93.49	2.24	97.76	4.21	95.79	0.69	99.31	1.92	98.08
ABCR1	8	94	2	98	4	96	2	98	2	98
ABCR2	6	92	4	96	6	94	3	97	4	96
ABCR3	9	91	6	94	7	93	4	96	10	90
2Metals+SDS	Ni(II)	Cd(II)	SDS	Ni(II)	Pb(II)	SDS	Pb(II)	Zn(II)	SDS	
EECR50	6.37	2.10	91.53	6.25	3.95	89.8	4.16	1.24	94.6	
ABCR1	6	2	92	6	4	90	4	1	95	
ABCR2	7	3	90	7	5	88	5	2	93	
ABCR3	5	2	93	11	2	87	2	4	94	
	Zn(II)	Cd(II)	SDS	Co(II)	Pb(II)	SDS	Co(II)	Cd(II)	SDS	
EECR50	1.27	2.21	96,52	1.84	4.14	94.0	1.88	2.20	95.93	
ABCRR1	2	2	96	4	2	94	3	3	94	
ABCR2	4	2	94	4	3	93	2	3	95	
ABCR3	4	3	93	3	1	95	2	2	96	

Key; Metal + SDS = Binary Mixtres, 2Met + SDS - Ternary

Table 3.2: Equieffect Concentration Ratio (EECR50) and Arbitrary Concentration Ratio (ABCR) of Quaternary, Quinary and Senary Mixtures for *S. marcescens* (SerEW01).

Mixtures	**Mixture Ratio (%)**													
3Met + SDS	Cd(II)	Zn(II)	Pb(II)	SDS	Cd(II)	Ni(II)	Pb(II)	SDS	Cd(II)	Co(II)	Pb(II)	SDS		
EECR50	2.12	1.22	4.07	92.59	2.01	6.12	3.87	87.99	2.11	1.80	4.05	92.04		
ABCR1	2	3	2	93	3	6	3	88	2	3	2	93		
ABCR2	3	3	3	91	4	6	4	86	3	3	3	91		
ABCR3	3	4	3	90	4	6	3	87	3	4	3	90		
4Met + SDS														
4Met + SDS	Cd(II)	Zn(II)	Pb(II	Co(II)	SDS	Cd(II)	Ni(II)	Pb(II	Zn(II)	SDS	Cd(II)	Zn(II)	Ni(II	Co(II)
EECR50	2.1	1.2	4	1.8	90.9	1.99	6.06	3.83	1.14	86.99	2.03	1.17	6.19	1.74
ABCR1	2	2	2	3	91	2	6	2	3	87	2	4	2	3
ABCR2	2	3	2	4	89	2	5	3	5	85	1	5	4	2
ABCR3	3	4	1	4	88	2	4	2	6	86	2	6	2	3
5Met + SDS	Cd(II)	Zn(II)	Pb(IIII	Co(II)	Ni(II)	SDS								
EECR50	1.96	1.12	3.76	1.68	5.95	85.53								
ABCR1	2	4	2	3	3	86								
ABCR2	2	4	2	3	4	85								
ABCR3	3	3	3	5	2	84								

Key: 3Met + SDS = Quternary Mixtures, 4Met + SDS = Quinary Mixtures, 5Met + SDS = Senary Mixtures

Table 3.3: Equieffect Concentration Ratio (EECR50) and Arbitrary Concentration Ratio (ABCR) of Binary and Ternary Mixtures for *A. seifertii*

Mixtures	**Mixture Ratio (%)**									
Metal+SDS	Ni(II)	SDS	Cd(II)	SDS	Pb(II)	SDS	Zn(II)	SDS	Co(II)	SDS
EECR50	3.93	96.07	0.21	99.79	3.93	96.07	1.30	98.70	0.93	99.07
ABCR1	3	97	1	99	3	97	2	98	1	99
ABCR2	5	95	2	98	5	95	10	90	3	97
ABCR3	10	90	4	96	6	94	4	96	7	93
2Metals+SDS	Ni(II)	Cd(II)	SDS	Ni(II)	Pb(II)	SDS	Pb(II)	Zn(II)	SDS	
EECR50	3.86	1.93	94.21	3.78	3.78	92.44	3.88	1.25	94.87	
ABCR1	5	2	93	4	3	93	4	1	95	
ABCR2	4	2	94	3	3	94	5	2	93	
ABCR3	6	3	91	5	4	91	2	8	90	
	Zn(II)	Cd(II)	SDS	Co(II)	Pb(II)	SDS	Co(II)	Cd(II))	SDS	
EECR50	1.30	0.20	98.50	0.89	3.89	95.22	0.91	2.0	97.10	
ABCR1	3	1	96	1	3	94	1	1	98	
ABCR2	1	1	98	3	2	95	1	2	96	
ABCR3	3	2	95	2	2	96	3	2	95	

Key: Metal + SDS = Binary Mixtures, 2Metals + SDS = Ternary Mixtures

Table 3.4: Equieffect Concentration Ratio (EECR) and Arbitrary Concentration Ratio (ABCR) of Quaternary, Quinary and Senary Mixtures for *A. seifertii*

Mixtures	**Mixture Ratio (%)**												
3Met + SDS	Cd(II)	Zn(II)	Pb(II)	SDS	Cd(II)	Ni(II	Pb(II	SDS	Cd(II)	Co(II)	Pb(II)	SDS	
EECR50	1.91	1.23	3.8	93.06	1.86	3.71	3.71	90.72	1.92	0.87	3.82	93.39	
ABCR1	2	3	2	93	3	4	2	91	2	2	2	94	
ABCR2	2	3	3	92	3	5	3	89	2	3	2	93	
ABCR3	3	4	2	91	3	4	3	90	2	4	2	92	
4Met + SDS	Cd(II)	Zn(II)	Pb(II)	Co(II)	SDS	Cd(II)	Ni(II)	Pb(II	Zn(II)	SDS	Cd(II)	Zn(II)	Ni(II
EECR50	1.89	1.22	3.77	0.86	92.25	1.84	3.67	3.67	1.19	89.64	1.89	1.22	3.78
ABCR1	1	2	1	3	93	2	3	1	4	90	1	3	2
ABCR2	2	3	2	2	91	3	3	2	4	88	1	2	2
ABCR3	2	4	2	2	90	1	4	3	3	89	2	2	3
5Met + SDS	Cd(II)	Zn(II)	Pb(II	Co(II)	Ni(II)	SDS							
EECR50	1.82	1.81	3.64	0.83	3.64	88.8							
ABCR1	1	2	2	3	3	89							
ABCR2	2	3	2	2	3	88							
BCR3	3	3	2	3	2	87							

Key: 3Met + SDS = Quternary Mixtures, 4Met + SDS = Quinary Mixtures, 5Met + SDS = Senary Mixtures

3.8.7.3.Design of SDS and metals mixture bioassay

The dehydrogenase activity assay was done using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) as the artificial electron acceptor, which was reduced to the purple-coloured MTT-formazan (MTTF). The assay was done in 2-ml volumes of nutrient broth-MTT medium (pH 7) supplemented with varying concentrations of SDS and Cd(II), Pb(II), Zn(II), Co(II) or Ni(II) in separate 15 ml screw-capped culture tubes. A 0.5 ml portion of x4-strength nutrient broth and requisite volumes of sterile deionized water and stock solutions of (10 or 50 mM) of the respective heavy metals and SDS were added to each tube in duplicates to obtain the different binary, ternary, quaternary, quinary ans senary mixtures of SDS+metal ions ratios. Thereafter, 0.1 ml each of 0.1% aqueous solutions of MTT and bacterial suspension were added into each tube. The final concentrations of the toxicants ranged from 0.05 to 3.0 mM. The controls consisted of the medium without SDS and heavy metals. The cultures were incubated at room temperature ($28 \pm 2^{o}C$) for 24 hours.

3.8.7.4. Extraction and quantification ofMTT-formazan

At the end of the incubation,the reaction was stopped by adding 4 ml of n-butanol and then shook for about 10 minutes. The MTT-formazan produced was extracted into the n-butanol. Absorbance of the extract was determined spectrophotometrically at 590nm (VIS Spectrophotometer 721D).

3.9. Data Analysis

3.9.1. Transformation of the dose-response data

The inhibition of dehydrogenase activity from each toxicity assessment was transformed relative to the mean control (SD < 5%) to a 0 to 100% scale as shown in Eq. 1. The normalized responses were generated as mean and their standard deviations from duplicate determinations.

$$R = \left[1 - \frac{T_A}{C_A}\right] X 100 \qquad (1)$$

Where R is the inhibition (%) of dehydrogenase activity, $C_A$is the absorbance ofMTTF-extract in the control experiment and $T_A$is absorbance of MTTF-extract in the test experiment with different concentrations of SDS and metal ion(s).

3.9.2. Determination of toxicity thresholds (EC_{50}s)

3.9.2.1. Non-hormetic model

The EC_{50} thresholds for the individual toxicants and their mixtures were determined by graphing and fittingthe dose-response data with 2-parameter logistic function(Eq. 2),implemented in Table curve 2-D software.

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

Where x is the concentration of toxicant, $EC_{50}$is the concentration of toxicant that inhibited dehydrogenase activity by 50% and b is the slope at EC_{50}.

3.9.2.2. Hormesis model

In the case of hormetic responses (stimulation of dehydrogenase activity at low concentrationsin in SDS, heavy metals or their mixtures), the EC_{50} values were determined by fitting the dose-response data to hormesis-model of Schabenberger*et al.*,(1999) (Eq. 3).

$$R = 100 - \left[\frac{100 - fx}{1 + \left[\frac{p}{100 - p} + \left\{\left(\frac{100}{100 - p}\right)\frac{fEC_p}{100}\right\}\right]\left(\frac{x}{EC_p}\right)^b}\right] \qquad (3)$$

Where *f* is the parameter describing the degree of hormetic response, p is the percentage decrease in response, EC_p is the concentration of the toxicant at a given p. the parameter b is no longer the slope at EC_{50}(Cedergreen *et al.,*2005).

3.9.3. Prediction of mixture toxicities

3.9.3.1. Concentration addition model

The toxicities of the mixtures can be determined from the toxicity of the individual component based on concentration addition (CA) model, if the relative composition of each component is quantitatively known. The concept of concentration addition assumes that the components of the mixture acts similarly against the test organism. The CA model can be written as (Berenbaum, 1985)

$$EC_{x(mix)} = \left[\sum_{i=1}^{n} \frac{\pi_i}{EC_{xi}} \right]^{-1} \quad (4)$$

Where *ECx*(mix) is the total concentration of the mixture that elicited *x*% effect, *ECxi* is the concentration of *i*th component that gave *x* effect when tested as an individual, *n* is the number of components, πi is the proportion of *i*th component in the mixture, such that the sum of πi = 1. Using Eq. 4, the toxicities of the mixtures were predicted as described elsewhere (Altenburger *et al*., 2000; Backhaus *et al*., 2000). The total concentration of each mixture that elicited 1 – 99% effects were calculated in steps of 1%. The resulting 99 concentration/effect pairs were plotted as a line chart giving a visualization of the predicted dose-response curve. First, the *ECx* for 1 – 99% was calculated for each component from the logistic dose-response model that fitted the individual dose-response data. Secondly, the *ECx* values were substituted in Eq. 3 to obtain the 1 – 99% EC*x*(mix) values for each mixture (Nweke *etal*. 2018).

In an n-component mixture, Eq. 4 for an EC_{50} can be substituted into Eqs. 2 and 3 to give Eqs. 5 and 6 respectively.

$$R = \frac{100}{1 + \left\{ \sum_{i=1}^{n} \frac{\pi i x}{EC_{50i}} \right\}^{b}} \quad (5)$$

$$R = 100 - \frac{100 + fx}{1 + \left[1 + \left\{ \frac{2f}{100\left(\sum_{i=1}^{n} \frac{\pi i}{EC_{50i}} \right)} \right\} \right] \left(\sum_{i=1}^{n} \frac{\pi i x}{EC_{50i}} \right)^{b}} \quad (6)$$

Where x is the total concentration of all the components in the mixyure and b is the average slpoe for individual components (Rider and LeBlanc, 2005).

3.9.3.2. Independent action model

The independent action (IA) or response addition model assumes that the components of a given mixture have different mode of action. The mathematical expression is as follows (Altenburger *et al*., 2000; Faust *et al*., 2003):

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} \left[1 - E(c_i) \right] \quad (7)$$

Where $E(c_{mix})$ represents the total effect or response (scaled from 0 to 1) of an *n*-component mixture, $c_i$is the concentration of the *i*th component and $E(c_i)$ is the effect or response of the individual component. The dose-response relationships $F_i$of the individual components were used to calculate their effects $E(c_i)$ as shown in Eq. 8 below (Backhaus *et al*., 2000).

$$E(c_{mix}) = 1 - \prod_{i=1}^{n}\left[1 - E(c_i)\right] = 1 - \prod_{i=1}^{n}\left[1 - F_i(c_i)\right] \qquad (8)$$

By expressing the concentrations of the individual components as fractions, π_i, of the total concentration, c_{mix}, the overall effect of any given mixture concentration can be calculated as:

$$E(c_{mix}) = 1 - \prod_{i=1}^{n}\left[1 - E(c_i)\right] = 1 - \prod_{i=1}^{n}\left[1 - F(\pi_i.c_{mix})\right] \qquad (9)$$

The total effect $E(c_{mix})$ of each mixture were calculated for c_{mix}values ranging from 0.1 – 9 mM and multiplied by 100, as shown in Eq. 10, to rescale the effect from 0 to 100%.

$$E(c_{mix}) = \left[1 - \prod_{i=1}^{n}\left[1 - F(\pi_{i.}.c_{mix})\right]\right] x100 \qquad (10)$$

To implement this, equation 2 was substituted into Eq. 10 for each metal ion as:

$$R = \frac{1}{1 + \left[\frac{\pi_i x}{EC_{50}}\right]^b} \qquad (11)$$

Thus, the independent action model as simplified by Nweke *etal*. (2018) is expression as (Eq. 11):

$$E(c_{mix}) = \left[1 - \prod_{i=1}^{n}\left[1 - \frac{1}{1 + \left(\frac{\pi_i x}{EC_{50i}}\right)^{bi}}\right]\right] \times 100 \qquad (12)$$

Where, $\pi_i x$ is the concentration of *i*th component in the mixture. The values of $EC_{50i}$and $b_i$as generated from Eq. 2 for individual metal ion and SDS were used. The effect of the mixture $E(C_{mix})$ at x ranging from 0 to 8 mM was calculated according to Eq. 12 using Microsoft Excel 2007.

The experimentally-derivedEC_{50S} for individual toxicants as well as for the four mixtures ratios in each mixture type were compared. Similarly, within each mixture ratio, the experimentally-derived EC_{50}, CA- and IA-predicted EC_{50S} were equally compared using Duncan post-hoc tests, implemented with SPSSStatistics 21 at $P<0.05$ level of significance.

3.9.4. Determination of the mixture effects

3.9.4.1. The toxic index (TI)

The Toxic Index (TI) of each mixture was calculated as sum of toxic units for all the components of the mixture (equation 10) (Nweke *et al*., 2018).

$$\mathrm{TI} = \sum_{i=1}^{n} \frac{C_i}{EC_{50i}} = \sum_{i=1}^{n} \frac{\pi_i EC_{50mix}}{EC_{50i}} \quad (13)$$

Where $C_i$is the concentration of the *i*th component in the mixture at the $EC_{50}$of the mixture (EC_{50mix}) and $EC_{50i}$is the concentration of the *i*th 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 *i*th component in the mixture. TI = 1 describes additivity, TI > 1 describes antagonistic interaction and TI < 1 describes synergistic interaction (Boillot and Perrodin, 2008).

3.9.4.2. Model deviation ratios (MDR)

The model deviation ratios (MDR) were calculated as the ratio of the predicted $EC_{50}$to the experimentally-derived EC_{50}. MDR greater than 1 indicated that the model underestimated

toxicity, while a value of less than 1 indicated that the model overestimated toxicity. MDR values ranging from 0.5 to 2 ($0.5 \leq MDR \leq 2$) indicated that the mixture was most likely to be additive (Petersen and Tollefsen, 2011; Li *et al*., 2014).

$$\mathbf{MDR} = \frac{\mathbf{Predicted\ EC_{50}}}{\mathbf{Observed\ EC_{50}}} \qquad \mathbf{(14)}$$

3.9.5. Isobolographic analysis

The estimated EC_{50}were used in subsequent determination of isoboles and isobolographic analysis of the binary mixture toxicity as described by Nweke *et al*. (2014). The concentrations of each component at EC_{50}(C_i) were calculated and used to compute the isoboles. The $C_i$values ($C_{iA}$and C_{iB}) for the components can be calculated by multiplying the proportion of individual component in the mixture by the $EC_{50}$of the mixture as in the numerator of Eq. 13**.**Triplicate isoboles were generated and plotted in an isobologram as described by (Boillot and Perrodin, 2008; Nweke *et al*., 2014). The straight line joining the $EC_{50}$of component A on one axis and $EC_{50}$of component B on the other axis is the line of additivity representing the additive effect of the mixture. When an isobole plotted in the isobologram is below or above the additivity line, the interaction is taken to be synergistic or antagonistic respectively.

CHAPTER FOUR

4.0 RESULTS

4.1.Physicochemical Characteristics of Otamiri River Water and Sediment

The physicochemical parameters of Otamiri river and sediment are shown in Table 4.1. From the table, the pH and temperature of the river water were 6.42 and 26.1^{o}C, while the sediment recorded pH of 5.40.Similarly, the phosphate contents of the river water and sediment were 0.032 mg/l and 18.41 mg/kg respectively. Conductivity recorded 115.8 µS/cm in the river, while total hardness was 0.32 mg/l. Turbidity, chloride, BOD and DO recorded 22.8 NTU, 1.08 mg/l, 5.0 and 9.8 mg/l respectively in Otamiri river water.

Cobalt, iron, copper, lead, cadmium, zinc, nickel and mercury were the heavy metals recoded in the river water and sediment.The Table 4.1 also showed that iron, zinc and mercury recorded the highest values; 19.818, 16.548 and 3.678mg/l in sediment and 1.972, 1.556 and 1.329mg/l in river water sample respectively. Cadmium recorded the least value (0.025mg/l) in sediment while cobalt was not detected in the river water sample. Among the anionic surfactants, sodium dodecyl sulfate (SDS) recorded the highest values in river water (0.100µg/l) and sediment (0.453µg/l), while Perfluorobutanesulfate was not detected in the river water. Sodium lauryl sulfate recorded the least value in the sediment sample (0.0018µg/l).

Table 4.1: Physicochemical Properties of Otamiri River and Sediment Samples

Parameter	**Sediment (mg/kg)**	**River (mg/l)**	**WHO Standard (Water)**
Cobalt	0.163	0.000	No guideline
Iron	19.818	1.972	No guideline
Copper	0.969	0.059	2mg/l
Lead	2.383	0.546	0.01mg/l
Cadmium	0.025	0.093	0.003mg/l
Zinc	16.548	1.556	3mg/l
Nickel	1.054	0.066	0.02mg/l
Mercury	3.678	1.329	0.001mg/l
pH	5.40	6.42	6.5-8.5
Phosphate	18.41	0.032	3.5
Temperature		26.1	None
Conductivity (µS/cm)		115.8	100
Total hardness		0.32	500
Turbidity (NTU)		22.8	5.0
Chloride		1.08	5mg/l
BOD		5.0	5.0
DO		9.8	10
Perfluorobutanesulfate	0.0142	0.000	
Sodium methyl sulfate	0.0532	0.060	
Ammonium lauryl sulfate	0.0303	0.070	
Sodium dodecyl sulfate	0.4531	0.100	
Sodium laureth sulfate	0.0018	0.070	

4.2.**Bacteriological Quality of Otamiri River Water and Sediment**

The biochemical characteristics and percentage occurrence of the bacterial isolates from the Otamiri river and sediment are shown in Tables 4.2 and 4.3**.**Five different bacterial genera were isolated from the water sample, with *Serratia marcescens* (SerEW01) (33.33%) recording the highest percentage occurrence (Plate III) while *Enterobacter*species and *Escherichiacoli* recorded the least (11.11%).In sediment sample, six bacterial genera were isolated, with *Acinetobacter seifertii* recording the highest percentage occurrence (42.10%) (Plate IV)and*Streptococcus* species recorded the least (5.30%).The 16S rRNA gene partial sequencing further confirm the identities of these preponderant bacterial isolates from the river water and sediment to be *S. marcescens* (SerEW01) (33.33%) and *A. seifertii* respectively, as shown in Figures 4.1 and 4.2.

Table 4.2: Biochemical Characteristics and % Occurrence of Bacterial Isolates from Otamiri River

No of positive in Sample	Morphological Characteristics on Nutrient Agar	Gram Reaction	Mot Test	Indol Test-	Spore Staining	Cat Test	Cit Test	Sugar Ferm Test S	B	G	H_2S	Bacterial isolated	% Occurrence of Isolates
4	Milkish raised non-mucoid colonies	Gram+ cocci	-	-	-	-	-	R	Y	+	-	*Staphylococcus*sp	22.2
2	Colourless large mucoid colonies	Gram- rods	-	-	-	-	+	Y	Y	+	-	*Enterobacter*sp	11.11
6	Whitish smooth mucoid colonies	Gram- rods	-	-	-	+	-	-	-	-	-	*Serratia marcescens* (SerEW01)	33.33
4	Milkish raised non mucoid colonies	Gram+ cocci	-	-	-	-	-	-	-	-	-	*Streptococcus*sp	22.22
2	Orange round colonies	Gram- rods	-	+	-	-	+	Y	Y	+	-	*Escherichia coli*	11.11

Key:+ = positive, - = negative,S= slope colouration, B = butt colouration, G = gas production., H_2S= Hydrogen sulphide production,Y= Yellowish colouration (acidic), Reddish pinkish colouration (alkaline production)

Table 4.3: Biochemical Characteristics of Bacterial Isolates from Otamiri Sediment

No of Positive Samples	Morph Characteristics on NA	Gram Stainn	Mot Test	Indol Test	Spore Stain	Cat Test	Cit Test	Sugar Ferm Test			H_2S	Bacterial isolated	% Occurrence of Isolates
								S	B	G			
1	Milkraised non-mucoid colonies	Gram+ rods	-	-	-	-	-	-	-	-	-	*Streptococci*sp	5.30
2	White big round colonies	Gram - rods	+	-	-	+	+	Y	Y	+	-	*Pseudomonas*sp	10.53
2	Colourless/milk fish colonies	Gram - cocci	-	-	-	+	+	-	-	-	-	*Klebsiella*sp	10.53
8	Pinkish raised non mucoid	Gram - rods	+	-	-	-	-	R	Y	-	-	*Acinetobacter seifertii*	42.10
3	Large flat colonies	Gram+rods	+	-	+	+	+	R	Y	-	-	*Bacillus*sp	15.80
3	Orange round colonies	Gram - rods	-	+	-	-	+	Y	Y	+	-	*Escherichia coli*	15.80

Key:**NA =** Nutrient Agar,**+** = positive, **-** = negative, S= slope colouration, B = butt colouration, G = gas production., H_2S= Hydrogen sulphide production, Y= Yellowish colouration (acidic), Reddish pinkish colouration (alkaline production)

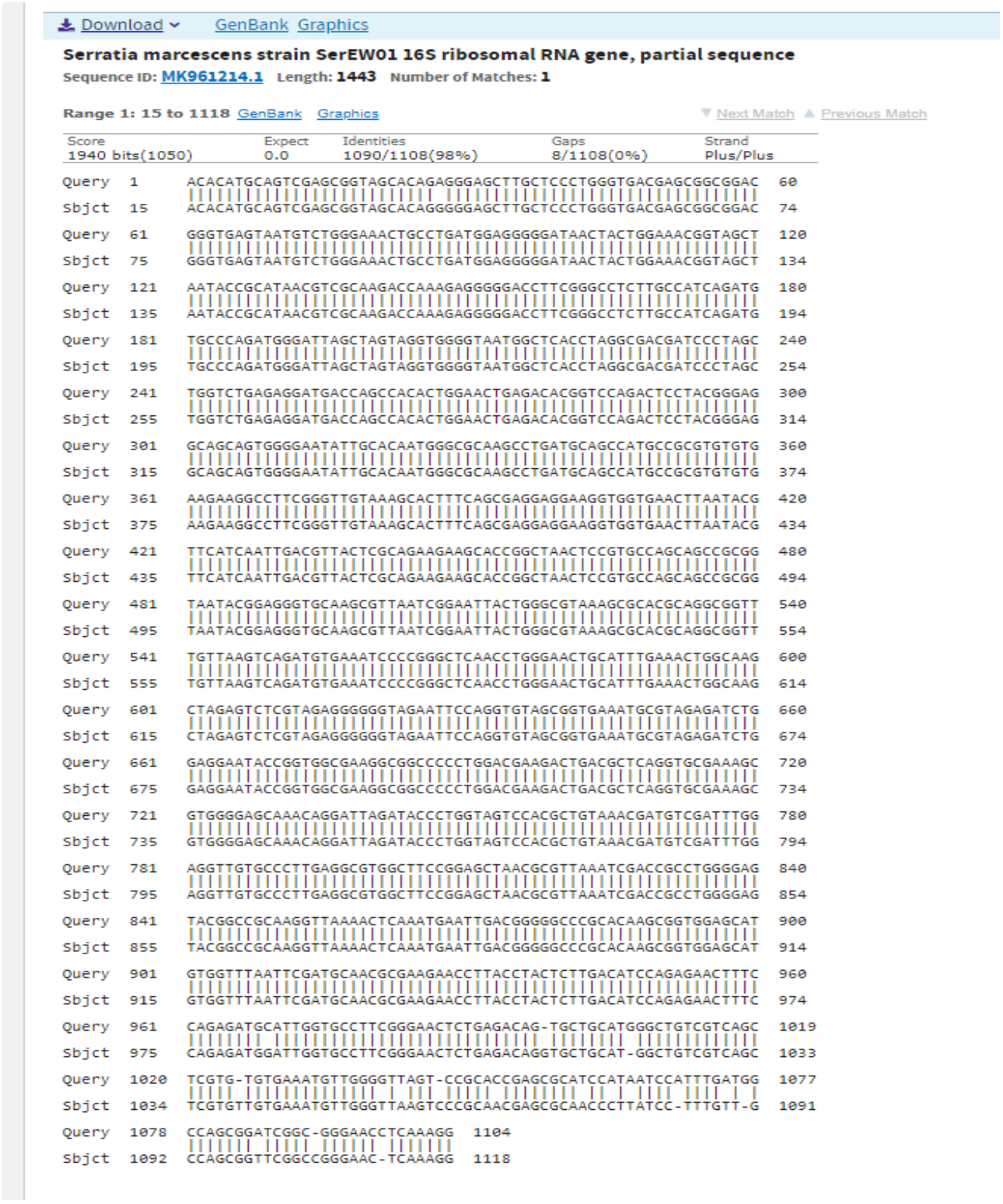


Figure 4.1. 16S rRNA partial gene sequencing of preponderant isolate from the river water,*Serratiamarcescens* (SerEW01).

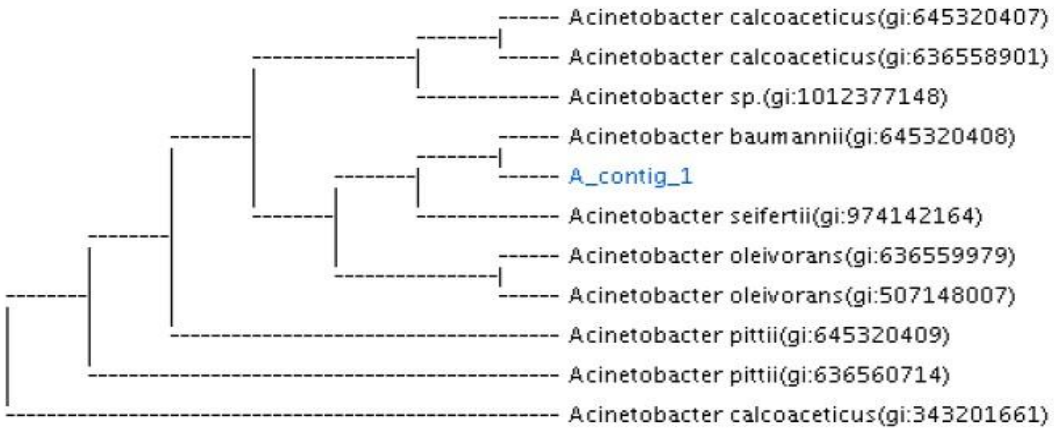


Figure 4.2. Phylogenetic tree showingthe result of 16S rRNA partial gene sequencingfor the sediment preponderant bacterium,*Acinetobacterseifertii*.

4.3.ToxicityAssays

4.3.1. Toxicity ofindividual toxicants

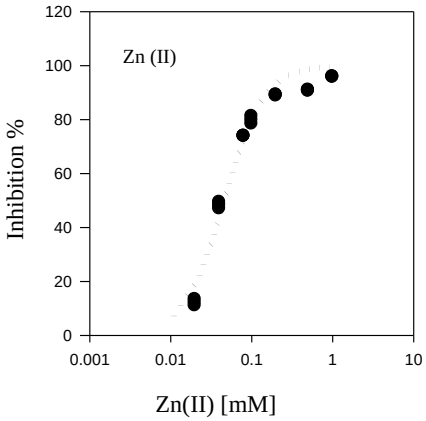
4.3.1.1.Toxicity of individual toxicants to*Serratiamarcescens (SerEW01)*

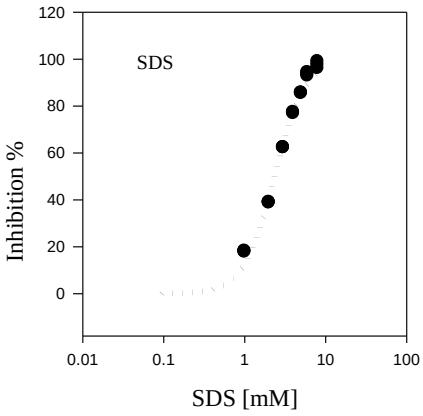
Table 4.4is the experimental toxicity thresholds (EC_{50}) of individual metal ions and SDS for *Serratiamarcescens* (SerEW01).The EC_{50S} of the toxicants ranged from 0.046 ± 0.003mM for Zn(II) to 2.329±0.092 mM for SDS.The Duncan test indicates that the EC_{50S} of the toxicants were significantly different from oneanother ($P < 0.05$)and the order of decreasing toxicity was Zn(II)>Cd(II)>Co(II)>Ni(II)>Pb(II)>SDS. The effects of the individual toxicants on the dehydrogenase activity of *S. marcescens* (SerEW01) as well as fit of the monotonic logistic model are shown in Figure 4.3. The response of the organism to the toxicity of the toxicants was dose-dependent. The toxicants progressively inhibited the dehydrogenase activity as the concentration increases, giving percentage inhibitions greater than 95% at 1 mM for Zn(II) and Ni(II), 0.5mM for Pb(II), Cd(II) and Co(II) as well as 8mM for SDS. The shapes of the dose-response curves are rather similar for SDS, Cd(II) and Co(II).

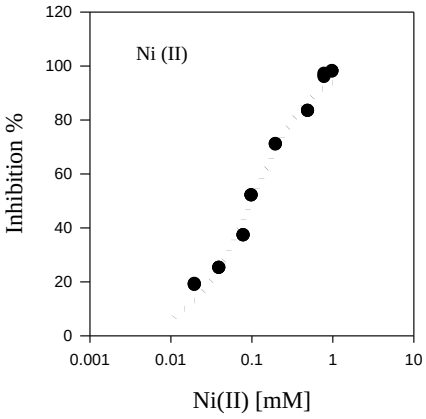
Table 4.4: Experimentally-derived Toxicity Thresholds (EC_{50}) of Individual Metals and SDS on *Serratia marcescens* (SerEW01)

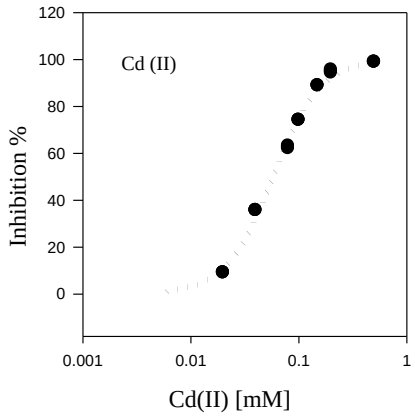
Toxicants	**Experimental EC_{50}(mM) †**
Ni(II)	0.100 ± 0.008a
Cd(II)	0.058 ± 0.002b
Pb(II)	0.113 ± 0.005c
Zn(II)	0.046 ± 0.003d
Co(II)	0.086 ± 0.002e
SDS	2.329 ± 0.092

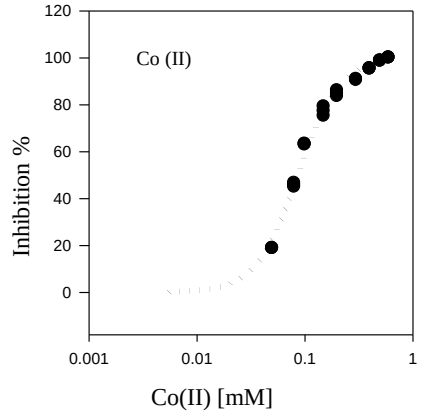
†The experimentally-derivedEC_{50} values of the toxicants are significantly different from each other ($P < 0.05$). Values are reported as Mean ± 1SD


Zn (II)
Inhibition %
Zn(II) [mM]
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10


SDS
Inhibition %
SDS [mM]
120
100
80
60
40
20
0
0.01
0.1
1
10
100


Ni (II)
Inhibition %
Ni(II) [mM]
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10


Cd (II)
Inhibition %
Cd(II) [mM]
120
100
80
60
40
20
0
0.001
0.01
0.1
1


Co (II)
Inhibition %
Co(II) [mM]
120
100
80
60
40
20
0
0.001
0.01
0.1
1

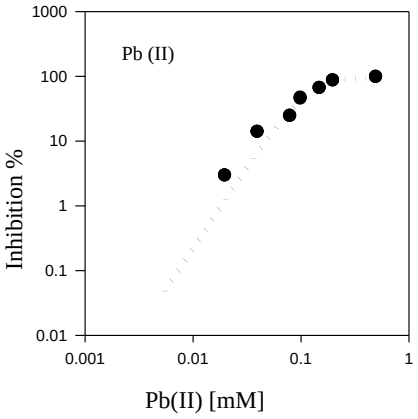

Pb (II)
Inhibition %
Pb(II) [mM]
1000
100
10
1
0.1
0.01
0.001
0.01
0.1
1

Figure 4.3: Inhibition of dehydrogenase activity of *Serratia marcescens* (SerEW01)by the individual toxicants.

4.3.1.2.Toxicity of individual toxicant to*Acinetobacter seifertii*

Table 4.5is the experimental toxicity thresholds (EC_{50S}) of individual metal ions and SDS for*Acinetobacterseifertii*. SDS with EC_{50} of 2.810 ± 0.140 mM had the least toxicity while cadmium with EC_{50} of 0.011 ± 0.000 mM had the highest toxicity. The results also showed that the EC_{50} values of all the toxicants were statistically different from one another ($P<0.05$) and the order of decreasing toxicity was Cd(II)>Co(II)>Zn(II)>Pb(II)>Ni(II)>SDS. The effects of the individual toxicants on the dehydrogenase activity of *A.seifertii*as well as the monotonic logistic model fitsare shown in Figure 4.4. The response of the organism to the toxicity of the toxicants was also dose-dependent. The toxicants progressively inhibited the dehydrogenase activity with increase in concentrations, giving percentage inhibitions greater than 95% at 0.4mM for Pb(II), 0.05 mM for Co(II), 0.08mM for Cd(II), 1mM for Zn(II) and 10mM for SDS. The dose-response patternwasalso similar for SDS and Ni(II)as well as for Cd(II) and Pb(II).

Table 4.5: Experimentally-derivedToxicity Thresholds (EC_{50}) of Individual Metals and SDS on *Acinetobacterseifertii*

Toxicants	**Experimental EC_{50}(mM) †**
Ni(II)	0.649 ± 0.053a
Cd(II)	0.011 ± 0.000b
Pb(II)	0.222 ± 0.005c
Zn(II)	0.075 ± 0.005d
Co(II)	0.041 ± 0.008e
SDS	2.810 ± 0.140

†The experimentally-derivedEC_{50} values of the toxicants are significantly different from each other (P <0.05). Values are reported as Mean ± 1SD

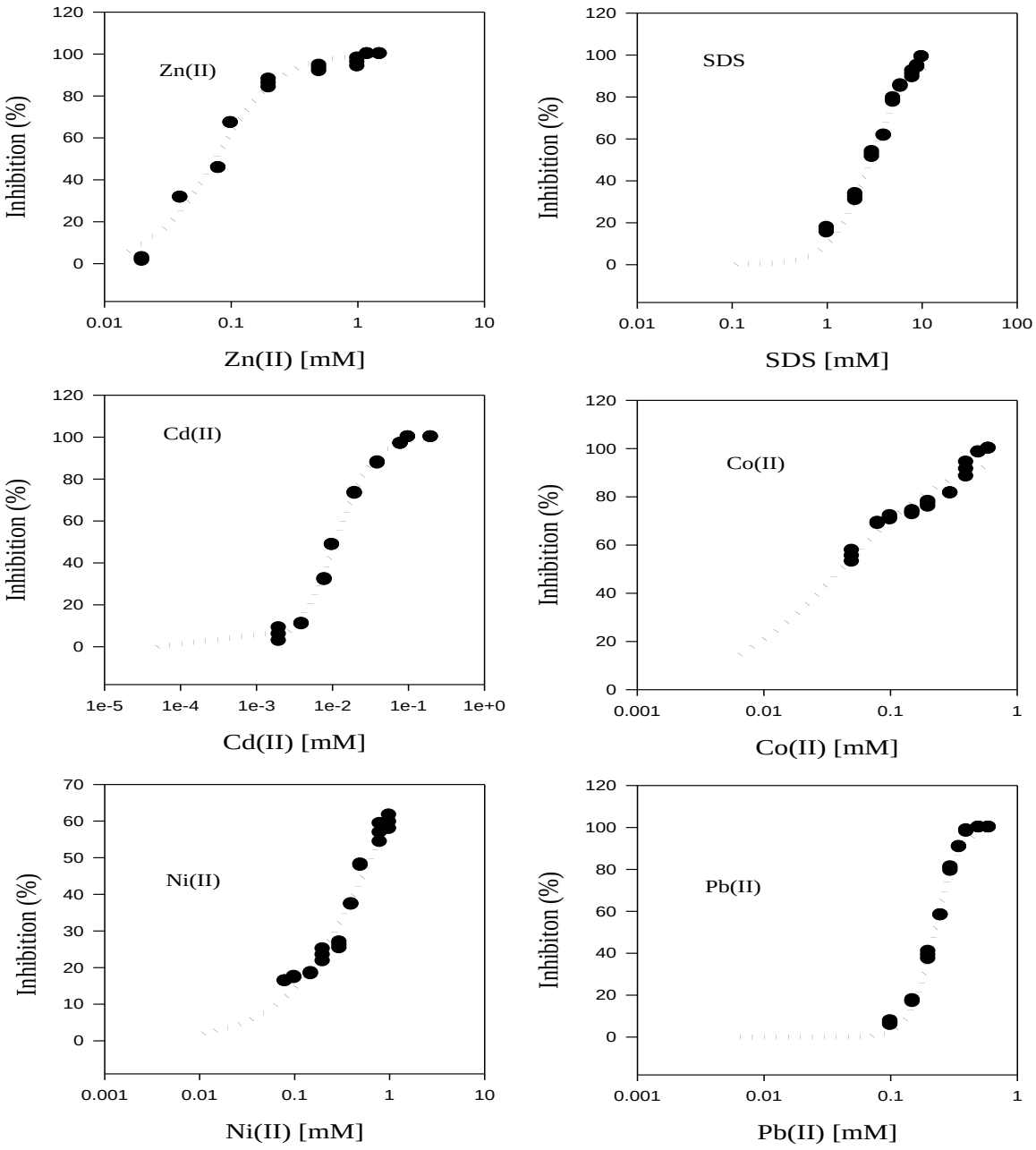


Figure 4.4: Inhibition of dehydrogenase activity of *Acinetobacter seifertii*by the individual toxicants.

4.3.2.Toxicity ofbinary mixtures

4.3.2.1.Toxicity of binary mixtures of SDS and metal ions to*S. marcescens (SerEW01)*

Table 4.6 is the experimentally-derived and predicted toxicity thresholds (EC_{50S}) of binary mixtures of metals and SDS on *S. marcescens* (SerEW01). The experimentally-derived EC_{50S}in the binary mixture of SDS and nickel showed that ABCR2 mixture ratio had the highest EC_{50}(0.314±0.013mM) while ABCR1 mixture ratio had the least EC_{50}(0.239±0.019mM). Also, in the binary mixture ype, the EECR50and ABCR2 mixture ratios were statistically different from ABCR1 and ABCR3.

InSDS+Cd(II) binary mixtures, the experimentally-derivedEC_{50S}ranged from 0.115±0.007mM (ABCR2) to 0.207±0.007mM(EECR50). The experimentally-derived$EC_{50S}$for different mixture ratios were statistically different from oneanother ($P < 0.05$). The same trend was observed in the binay mixtures of SDS + Zn(II), and SDS and Co(II). InSDS + Pb(II) binary mixtures, there was no statistical difference between the experimentally-derived EC_{50} of EECR50 and ABCR1,as well as between ABCR2 and ABCR3. In all binary mixture types, the experimentally-derived EC_{50S}, as well as those predicted based on CA- and IA-models,were statistically different from one another ($P < 0.05$), for all mixture ratios.

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on*S.marcescens* (SerEW01)are shown in Table 4.7. The toxic index (TI) values ranged from 0.123±0.002 to 0.543±0.007, while model deviation ratio (MDR) ranged from 1.839±0.028 to 10.771 ±0.445. At all the tested mixture ratios, the metals and SDS binary mixtures were synergistic in their actionon the bacterium.

The experimental dose-response relationships of the binary mixturesas well as the predictions made from CA and IA models for*S. marcescens* (SerEW01)are shown in Figures 4.5-4.9.In SDS92%+Ni(II)8% (ABCR2) and SDS91%+Ni(II)9% (ABCR3) mixture ratios, both CA and IA-models slightly overestimated the toxicities at low concentrations while under- estimating at higher concentrations. In other SDS+Ni(II) mixture ratios, the models predicted slightly lower toxicities than the experimentally-derived data would suggest,even at lower concentrations (Figure 4.5).Also, both models predicted similar toxicities for the binary mixtures, especially for SDS + Ni(II) mixtures, as their dose-response curves were almost superimposed. In SDS+Cd(II), SDS + Pb(II) and SDS + Co(II) binary mixtures,inhibition of dehydrogenase

activity took place even at low concentrations (Figures4.6, 4.7 and 4.9). Both CA and IA models grossly underestimated the mixture toxicities than the experimentally-derived data would suggest in most binary mixtures.In addition, in SDS + Co(II) mixture type, the 50% equieffect mixture ratio (SDS98.08% + Co(II)1.92%) was stimulatory against *S. marcescens* (SerEW01), at low concentrations and inhibitory at higher concentration. In SDS+Zn(II) mixtures, the models slightly predicted lower toxicities, especially for SDS99%+Zn(II)1% (ABCR1) and SDS96%+Zn(II)4% (ABCR3) mixture ratios (Figure 4.8).

The isobolographic analyses of the binary mixtures based on the EC_{50S} are shown in Figure 4.10. The isobologram indicated synergistic effect of all metals and SDS binary mixtureratios on the dehydrogenase activity. This observation was corroborated by the toxic index and model deviation ratio values as shown in Table 4.7.

Table 4.6: Experimentally-derived and Predicted Toxicity Thresholds (EC_{50}) of Binary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

Toxicant Binary Mixtures	EC_{50} (mM)‡ +		
	Experimental†	CA- Predicted	IA- Predicted
SDS + Ni(II)			
SDS 93.49% + Ni 6.51% (EECR50)	0.290± 0.016b*	0.952 ± 0.064**	1.147 ± 0.027***
SDS 94% + Ni 6% (ABCR1)	0.239 ± 0.019a*	0.998 ± 0.066**	1.199 ± 0.026***
SDS 92% + Ni 8% (ABCR2)	0.314 ± 0.013b*	0.838 ± 0.059**	1.012 ± 0.032***
SDS 91% + Ni 9% (ABCR3)	0.243 ± 0.010a*	0.776 ± 0.055**	0.936 ± 0.035***
SDS + Cd(II)			
SDS 97.76% + Cd 2.24% (EECR50)	0.207 ± 0.007a*	1.241 ± 0.046**	1.640 ± 0.014***
SDS 98% + Cd 2% (ABCR1)	0.176 ± 0.009b*	1.306 ± 0.049**	1.720 ± 0.016***
SDS 96% + Cd 4% (ABCR2)	0.115 ± 0.007c*	0.907 ± 0.033**	1.188 ± 0.012***
SDS 94% + Cd 6% (ABCR3)	0.155 ± 0.008d*	0.695 ± 0.025**	0.880 ± 0.014***
SDS + Pb(II)			
SDS 95.79% + Pb 4.21% (EECR50)	0.251 ± 0.012a*	1.276 ± 0.053**	1.764 ± 0.009***
SDS 96% + Pb 4% (ABCR1)	0.249 ± 0.007a*	1.305 ± 0.055**	1.804 ± 0.009***
SDS 94% + Pb 6% (ABCR2)	0.193 ± 0.010b*	1.070 ± 0.045**	1.690 ± 0.206***
SDS 93% + Pb 7% (ABCR3)	0.201 ± 0.012b*	0.982 ± 0.042**	1.331 ± 0.015***
SDS + Zn (II)			
SDS 98.70% + Zn 1.30% (EECR50)	0.661 ± 0.015a*	1.419 ± 0.077**	1.799 ± 0.010***
SDS 99% + Zn 1% (ABCR1)	0.725 ± 0.017b*	1.560 ± 0.081**	1.941 ± 0.013***
SDS 98% + Zn 2% (ABCR2)	0.310 ± 0.011c*	1.173 ± 0.068**	1.518 ± 0.017***
SDS 96% + Zn 4% (ABCR3)	0.426 ± 0.021d*	0.784 ± 0.050**	0.995 ± 0.032***
SDS + Co(II)			
SDS 98.08% + Co 1.92% (EECR50)	0.303 ± 0.011a*	1.554 ± 0.056**	2.042 ± 0.026***
SDS 98% + Co 2% (ABCR1)	0.188 ± 0.010b*	1.535 ± 0.058**	2.022 ± 0.024***
SDS 96% + Co 4% (ABCR2)	0.231 ± 0.010c*	1.142 ± 0.039**	1.547 ±0.006***
SDS 90% + Co 10% (ABCR3)	0.149 ± 0.009d*	0.649 ±0.024**	0.811 ± 0.012***

Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other (P <0.05).

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other (P <0.05).

Table 4.7: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Binary Mixtures on*S. marcescens* (SerEW01)

		MDR[+]		
Metal+SDS Mixtures	**Toxic Index (TI) [+]**	**CA**	**IA**	**Effect**
SDS + Ni(II)				
SDS 93.49% + Ni(II) 6.51% (EECR-50)	0.305 ± 0.004	3.281± 0.040	3.959 ± 0.125	Synergistic
SDS 94% + Ni(II) 6% (ABCR1)	0.239 ± 0.003	4.179 ± 0.056	5.034 ± 0.294	Synergistic
SDS 92% + Ni(II) 8% (ABCR2)	0.375 ± 0.011	2.671 ± 0.080	3.228 ± 0.027	Synergistic
SDS 91% + Ni(II) 9% (ABCR3)	0.314 ± 0.009	3.188 ± 0.089	3.846 ± 0.027	Synergistic
SDS + Cd(II)				
SDS 97.76% + Cd(II) 2.24% (EECR-50)	0.167 ± 0.001	5.994 ± 0.020	7.926 ± 0.200	Synergistic
SDS 98% + Cd(II) 2% (ABCR1)	0.135 ± 0.02	7.425 ± 0.103	9.785 ± 0.409	Synergistic
SDS 96% + Cd(II) 4% (ABCR2)	0.127 ± 0.003	7.900 ± 0.194	10.349±0.530	Synergistic
SDS 94% + Cd(II) 6% (ABCR3)	0.223 ± 0.004	4.489 ± 0.071	5.682 ± 0.203	Synergistic
SDS + Pb(II)				
SDS 95.79% + Pb(II) 4.21% (EECR-50)	0.197 ± 0.002	5.077 ± 0.041	7.031 ± 0.320	Synergistic
SDS 96% + Pb(II) 4% (ABCR1)	0.191 ± 0.003	5.240 ± 0.071	7.247 ± 0.174	Synergistic
SDS 94% + Pb(II) 6% (ABCR2)	0.181 ± 0.001	5.536 ± 0.039	8.727 ± 0.757	Synergistic
SDS 93% + Pb(II) 7% (ABCR3)	0.205 ± 0.004	4.887 ± 0.085	6.633 ± 0.324	Synergistic
SDS + Zn(II)				
SDS 98.70% + Zn(II) 1.30% (EECR-50)	0.466 ± 0,015	2.146 ± 0.067	2.723 ± 0.048	Synergistic
SDS 99% + Zn(II) 1% (BCR1)	0.465 ± 0.013	2.152 ± 0.062	2.680 ± 0.045	Synergistic
SDS 98% + Zn(II) 2% (BCR2)	0.264 ± 0.006	3.785 ± 0.091	4.905 ± 0.119	Synergistic
SDS 96% + Zn(II) 4% (ABCR3)	0.543 ± 0.007	1.839 ± 0.028	2.337 ± 0.040	Synergistic
SDS + Co(II)				
SDS 98.08% + Co(II) 1.92% (EECR-50)	0.195 ± 0.000	5.129 ± 0.006	6.743 ± 0.159	Synergistic
SDS 98% + Co(II) 2% (ABCR1)	0.123 ± 0.002	8.168 ± 0.129	10.771 ± 0.445	Synergistic
SDS 96% + Co(II) 4% (ABCR2)	0.202 ± 0.002	4.939 ± 0.057	6.694 ± 0.278	Synergistic
SDS 90% + Co(II) 10%(ABCR3)	0.230 ± 0.006	4.371 ± 0.100	5.462 ± 0.238	Synergistic

[+]Values are reported as Mean ± 1SD

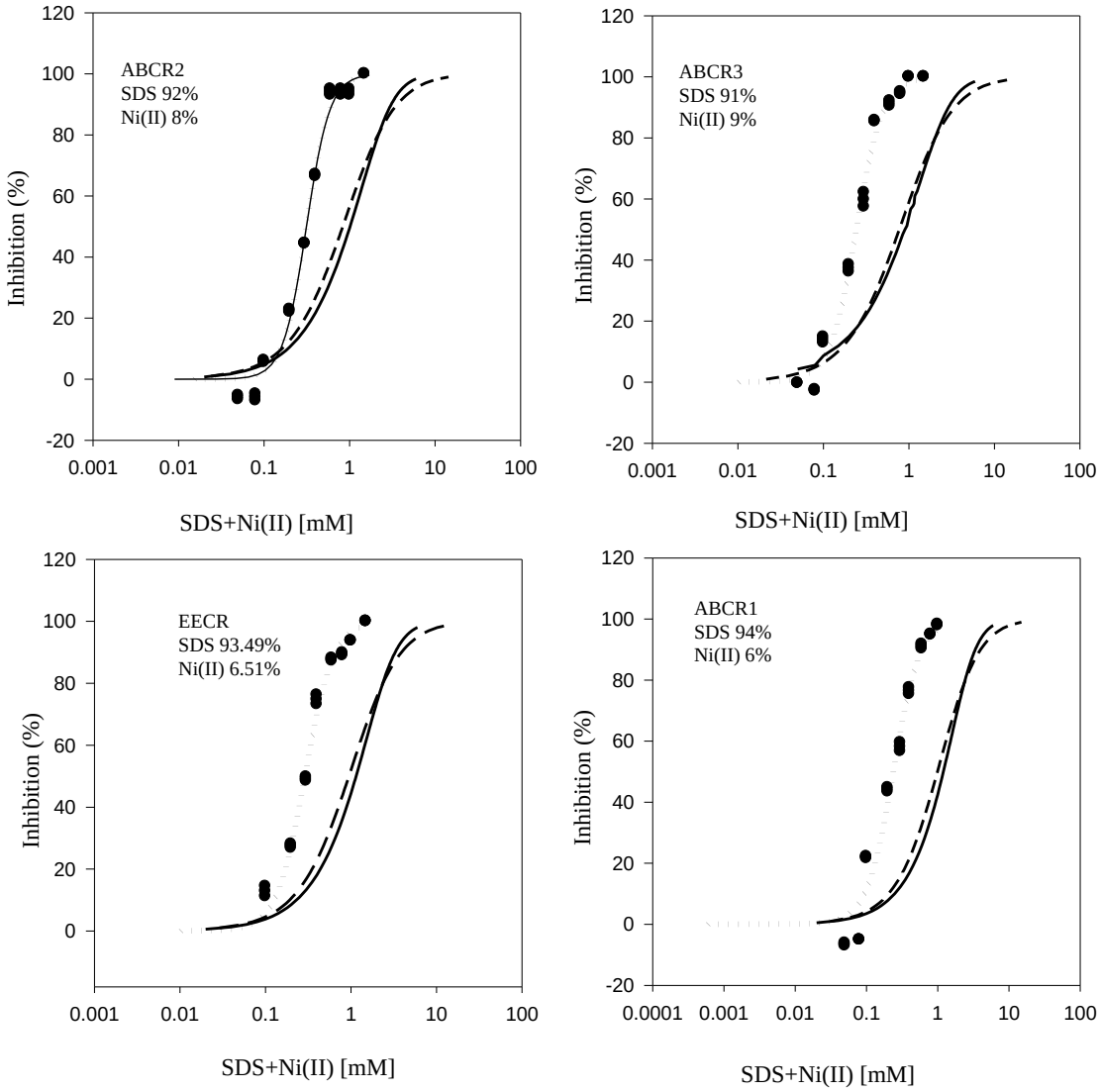


Figure 4.5: Experimental and predicted inhibitory effects of binary mixtures ofSDS and nickel ions on*S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

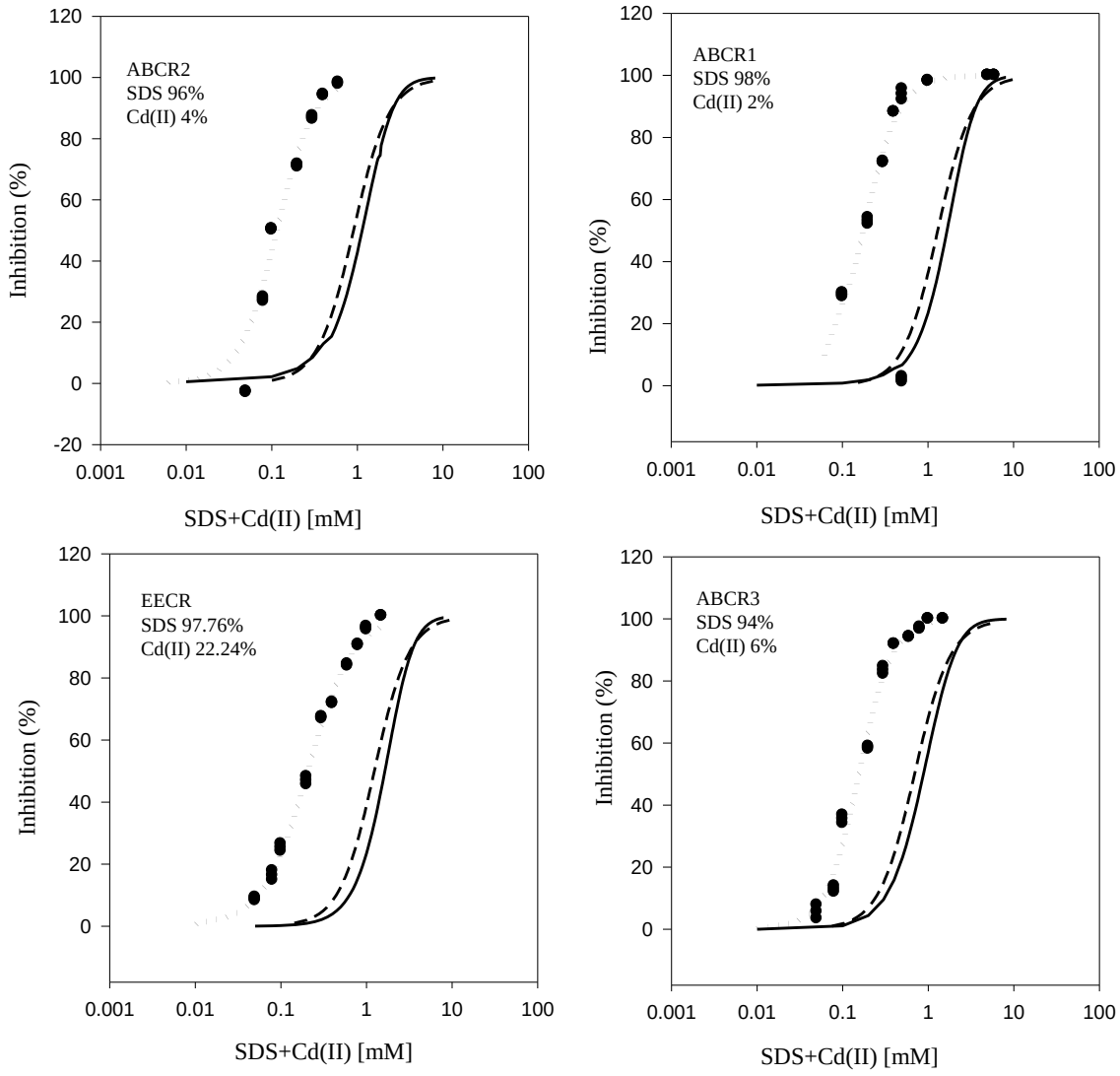


Figure 4.6: Experimental and predicted inhibitory effects of binary mixtures ofSDS and cadmium ions on*S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and dotted lines represent toxicities predicted from the concentration addition and the independent action models.

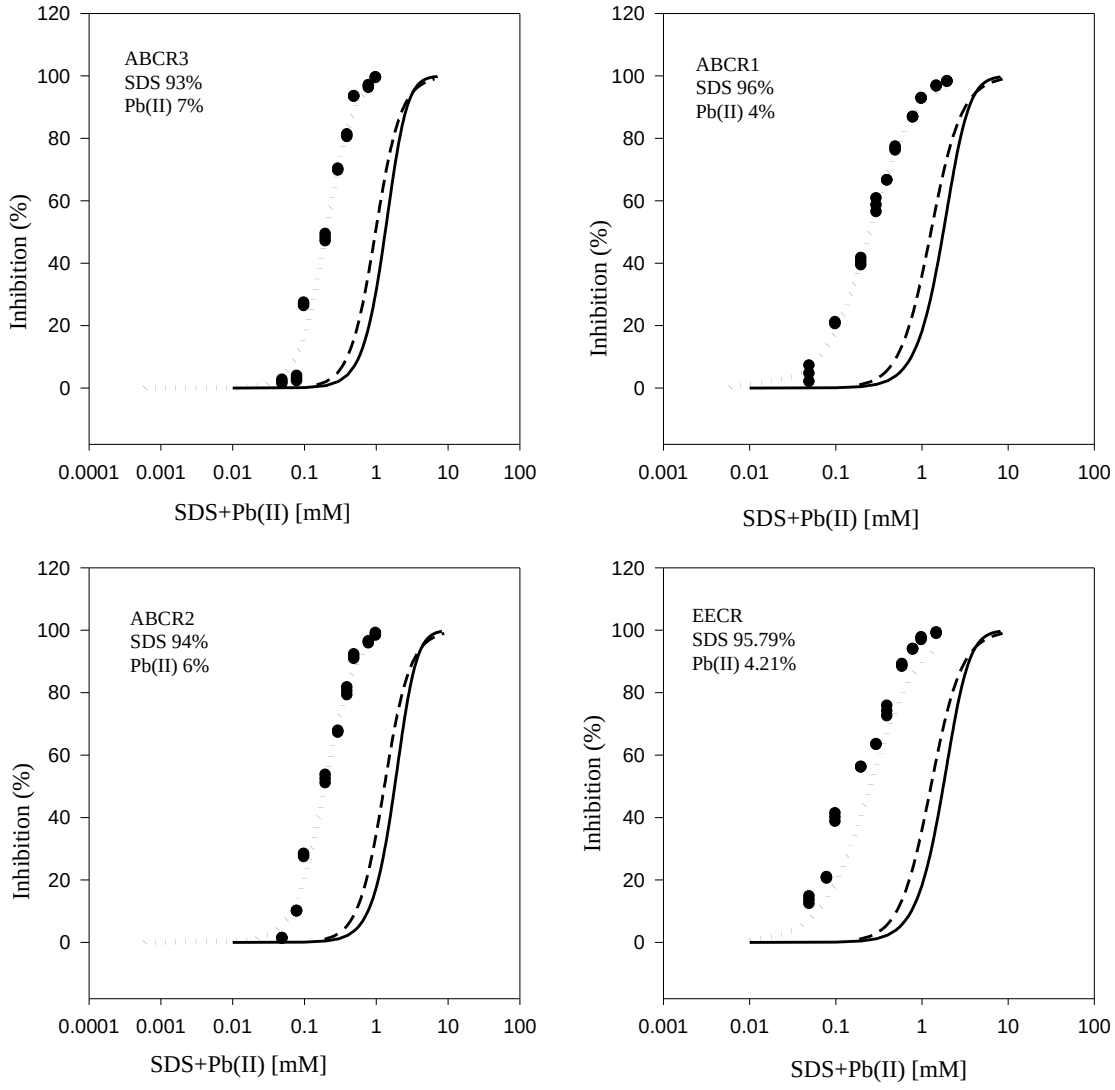


Figure 4.7: Experimental and predicted inhibitory effects of binary mixtures ofSDS and lead ions on*S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

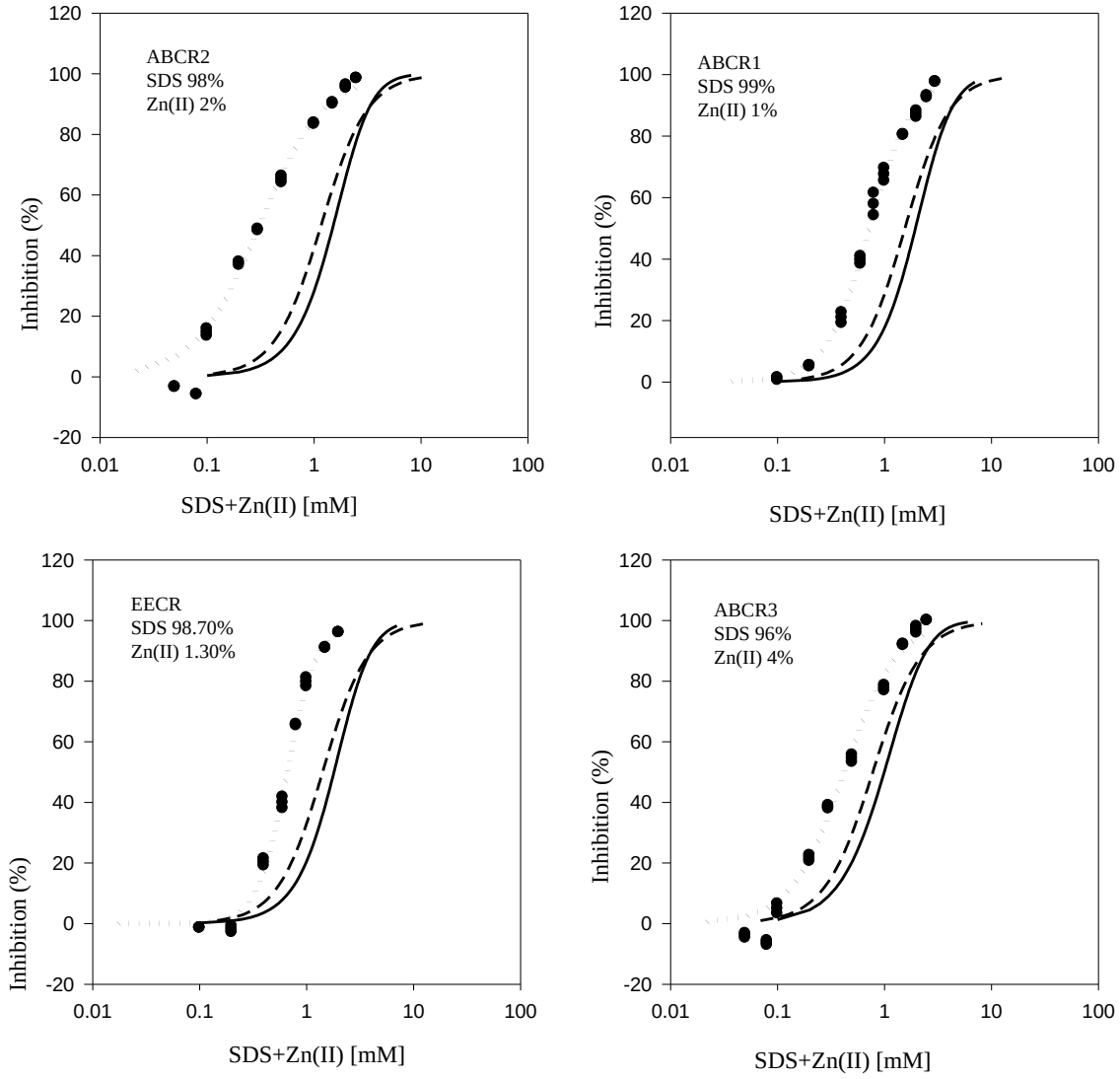


Figure 4.8: Experimental and predicted inhibitiory effects of binary mixtures ofSDS and zinc ions on*S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

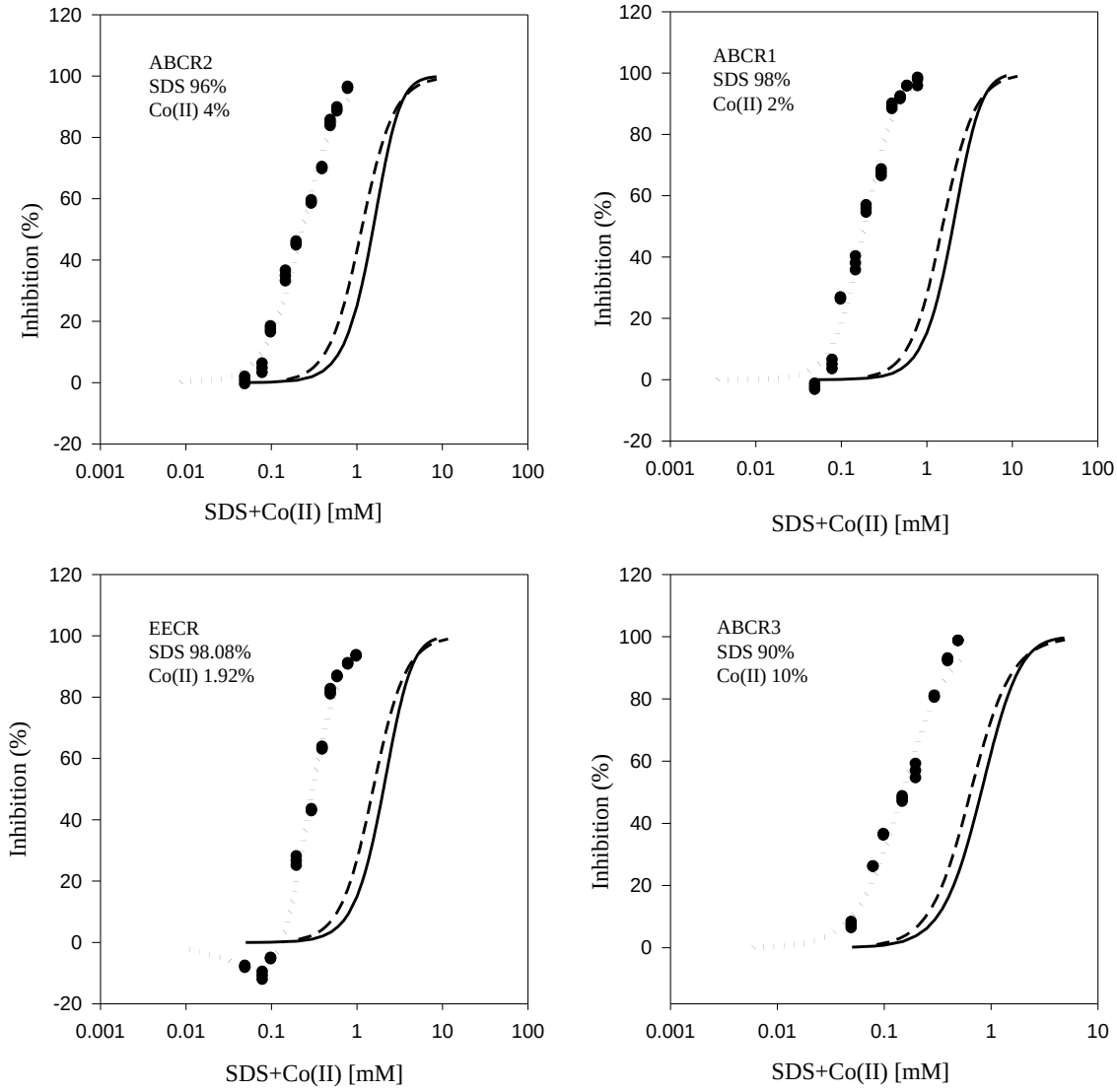


Figure 4.9: Experimental and predicted inhibitory effects of binary mixtures ofSDS and cobalt ionson *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq.2) or hormetic model (Eqn 3). Dashed and dotted lines represent toxicities predicted from the concentration addition and the independent action models.

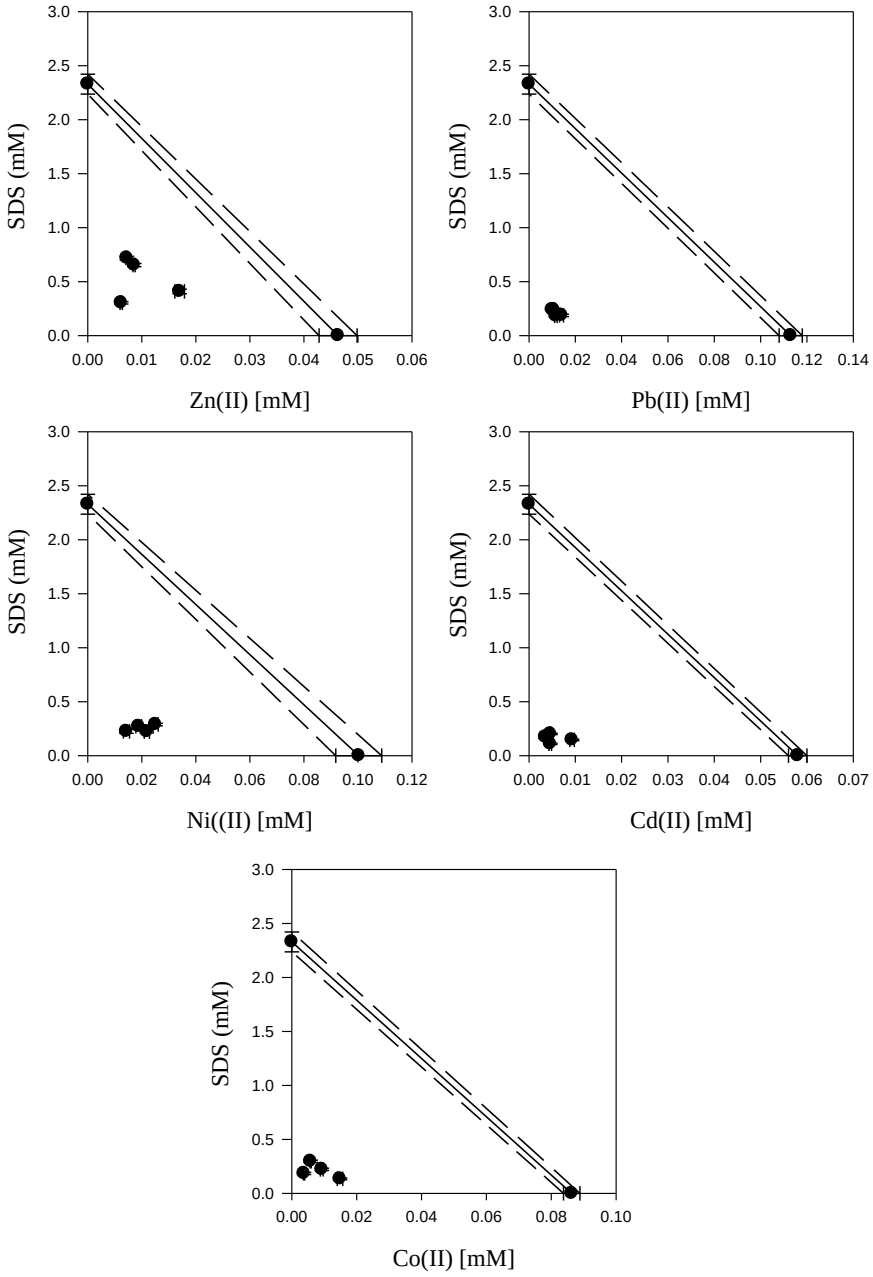


Figure 4.10: The EC_{50}isobole representation for SDS and metal ions and their mixtures tested against dehydrogenase activity in*S. marcescens* (SerEW01). The thick dots represent the standard deviation of the 95% confidence interval of the values. The solid and dashed lines represent additivity line and its 95% confidence belt.

4.3.2.2.Toxicity of binary mixtures of SDS and metals to*A. seifertii*

Table 4.8is the experimentally-derived and predicted toxicity thresholds (EC_{50}) of binary mixtures of metals and SDS on *A.seifertii.* In SDS+Ni(II) binary mixtures, the experimentally-derivedEC_{50S}ranged from 0.343±0.014mM (ABCR3)to 1.243±0.070mM (ABCR1) mixture ratios respectively. All the experimentally-derived EC_{50S} were significantly different from one another. In all mixture ratios of SDS + Ni(II) mixture type, there was no statistical difference betweenEC_{50S} predicied on the basis of CA-and IA-models but both were however statistically different from the experimentally-derived EC_{50S} ($P<0.05$). Similar trend was observed in the predicted EC_{50S} in SDS+Co(II) binary mixtures as well as inABCR3 mixture ratio of SDS + Cd(II) mixture type. Inaddition, in the binary mixtures of SDS +Cd(II), ABCR2 mixture ratio was the most toxic (0.283 ±0.006 mM), while EECR50mixture ratio was the least (0.996 ± 0.047 mM).

In SDS+Pb(II)binary mixtures, the experimentally-derived EC_{50S} ranged from 0.202±0.014mM(ABCR2) to 0.352±0.060mM (EECR50). Also, only ABCR2 mixture ratio hadexperimentally-derivedEC_{50}that was significantly different from the other mixture ratios. In the binary mixture of SDS +Zn(II), there was no significant difference between the experimentally-derived $EC_{50S}$for ABCR2 and ABCR3 mixture ratios. However, inall mixture ratios of SDS + Zn(II) and SDS + Pb(II) binary mixtures, experimentally-derived$EC_{50S}$and EC_{50S}predicted on the basis of CA- and IA-models were statistically different from one another ($P <0.05$).

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on*A.seifertii*are shown in Table 4.9. The toxic index (TI) values ranged from 0.114±0.004 to 1.805 ± 0.122,while model deviation ratio (MDR) ranged from 0.556±0.038 to 8.796 ± 0.293

for CA and 0.606 ± 0.045 to 13.275 ± 0.660 for IA.However, in all mixture ratiostested, the metals+SDS binary mixtures were synergistic in their actions against the bacteruim, exceptABCR3 (SDS96%+Cd(II)4%)that was antagonistic.

The experimental dose-response relationships of the binary mixtures, as well as the predictions made from CA and IA-models on*A.seifertii*are shown in Figures 4.11-4.15. In Figure 4.11, ABCR1 (SDS 97% + Ni(II) 3%) mixture ratios showed biphasic relationship within the concentration range of 0.062 to 0.5 mM while ABCR2 mixture ratio showed weak hormesis. In the other mixture ratios, the models correctly predicted the toxicities at low concentrations, while underestimation of the mixture toxicties occured at high concentration**.**Furthermore, both CA and IA models predicted identical toxicities for binary mixtures, especially inall SDS+Ni(II)and SDS + Co(II) mixture ratios, as well as ABCR3 (SDS 96% + Ni(II)4%)in SDS+Cd(II) mixtures, as their dose-response curves were almost superimposed.

In SDS+Cd(II) mixtures, inhibition of dehydrogenase activity took place even at low concentrations (Figure 4.12).In all its mixture ratios, both models predicted significantly lower toxicities than the experimentally-derived data, except for ABCR3 (SDS96%+Cd(II)4%) mixture ratio, where the models slightly overestimated the binary mixture toxicity, as reflected in Table 4.9. In SDS+Pb(II) mixtures, the models also grossly underestimated the toxicities relative to the experimentally-derived data and was toxic even at low concentrations (Figure 4.13).In Figure 4.14, the SDS+Zn(II) mixtures had biphasic effect on the dehydrogenase activity of *A.seifertii*The mixtures exhibited hormesis at low concentrations up to 0.1 mM for ABCR1and ABCR3mixture ratios, 0.09 and 0.3mM for ABCR2 and EECR50 respectively.Above these hormeticconcentration ranges, the mixture progressively inhibited the dehydrogenase activity of *A.seifertii***,** reaching 95% at 0.8mM for ABCR2, 96% at 2.5mM for

EECR50, 97% at 2.5mM for ABCR1 and 98% at 1.5mM for ABCR3mixture ratios.The inhibitory effects of SDS + Co(II) mixtures are shown in Figure 4.15. In ABCR1 mixture ratio, the models correctly predicted the experimentally-derived data at low concentrations. The EECR50 mixture ratio was also hormetic at low concentrations of upto 0.12 mM, whereas in the other two mixture ratios; both models predicted slightly higher toxicities at low concentrations. In addition, in all SDS+Co(II) mixtures, as the concentrations increased, both models slightly underestimated the toxicities.

The isobolographic analyses of the binary mixtures based on the EC_{50}values are shown in Figure 4.16. The isobologram indicated synergistic effect in all binary mixtures of SDS+metal ions, except ABCR3 mixture ratio of SDS+Cd(II) mixture, that was antagonistic. This observation was corroborated by the toxic index and model deviation ratio values as shown in Table 4.9.

Table 4.8: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Binary Mixtures of Metals and SDS on *A.seifertii*

	EC_{50} (mM)‡ [+]		
Toxicant Binary Mixtures	**Experimental†**	**CA-Predicted**	**IA- Predicted**
SDS + Ni(II)			
SDS 96.07% + Ni(II) 3.39% (EECR50)	0.939 ± 0.041a*	2.485 ± 0.129**	2.489 ± 0.037**
SDS 97% + Ni(II) 3% (ABCR1)	1.243 ± 0.070b*	2.555 ± 0.130**	2.549 ± 0.046**
SDS 95% + Ni(II) 5% (ABCR2)	0.816 ± 0.030c*	2.409 ± 0.128**	2.425 ± 0.029**
SDS 90%+Ni(II) 10% (AB CR3)	0.343 ± 0.014d*	2.108 ± 0.122**	2.186 ± 0.014**
SDS + Cd(II)			
SDS 99.79% + Cd(II) 0.21% (EECR50)	0.996 ± 0.047a*	1.850 ± 0.086**	2.286 ± 0.040***
SDS 99% + Cd(II) 1% (ABCR1)	0.524 ± 0.037b*	0.810 ± 0.039**	0.999 ± 0.025***
SDS 98% + Cd(II) 2% (ABCR2)	0.283 ± 0.006c*	0.473 ± 0.023**	0.547 ± 0.022***
SDS 96% + Cd(II) 4% (ABCR3)	0.467 ± 0.05b*	0.258 ± 0.013**	0.281 ± 0.013**
SDS + Pb(II)			
SDS 96.07% + Pb(II) 3.93% (EECR50)	0.352 ± 0.060a*	1.926 ± 0.073**	2.774 ± 0.052***
SDS 97% + Pb(II) 3% (ABCR1)	0.294 ± 0.018a*	2.081 ± 0.082**	2.857 ± 0.113***
SDS 95% + Pb(II) 5% (ABCR2)	0.202 ± 0.014b*	1.774 ± 0.064**	2.675 ± 0.053***
SDS 94% + Pb(II) 6% (ABCR3)	0.295 ± 0.017a*	1.652 ± 0.058**	2.628 ± 0.131***
SDS + Zn (II)			
SDS 98.70% + Zn(II) 1.30% (EECR50)	0.921 ± 0.012a*	1.909 ± 0.106**	2.674 ± 0.053***
SDS 98% + Zn(II) 2% (ABCR1)	0.582 ± 0.038b*	1.628 ± 0.095**	2.038 ± 0.013***
SDS 90% + Zn(II) 10% (ABCR2)	0.329 ± 0.019c*	0.607 ± 0.041**	0.714 ± 0.036***
SDS 96% + Zn(II) 4% (ABCR3)	0.362 ± 0.013c*	1.146 ± 0.072**	1.442 ± 0.025***
SDS + Co(II)			
SDS 99.07% + Co(II) 0.93% (EECR50)	0.580 ± 0.033a*	1.720 ± 0.182**	1.834± 0.197**
SDS 99% + Co(II) 1% (ABCR1)	0.464 ±0.031b*	1.671 ± 0.181**	1.769± 0.025**
SDS 97% + Co(II) 3% (ABCR2)	0.450 ± 0.017b*	0.926 ± 0.137**	1.044 ± 0.404**
SDS 93% + Co(II) 7% (ABCR3)	0.176 ± 0.005c*	0.490 ± 0.084**	0.547± 0.696**

[+]Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other (P <0.05).

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other (P <0.05).

Table 4.9: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Binary Mixtures on*A.seifertii*

Metal+SDS Mixtures	Toxic Index (TI) [+]	MDR[+]		Effect
		CA	IA	
SDS + Ni(II)				
SDS 96.07% + Ni(II) 3.93% (EECR50)	0.378 ± 0.003	2.646 ± 0.023	2.654 ± 0.076	Synergistic
SDS 97% + Ni(II) 3% (ABCR1)	0.486 ± 0.003	2.056 ± 0.011	2.054 ± 0.079	Synergistic
SDS 95% + Ni(II) 5% (ABCR2)	0.339 ± 0.006	2.951 ± 0.048	2.974 ± 0.076	Synergistic
SDS 90% + Ni(II) 10% (ABCR3)	0.163 ± 0.003	6.136 ± 0.098	6.373 ± 0.239	Synergistic
SDS + Cd(II)				
SDS 99.79% + Cd(II) 0.21% (EECR-50)	0.538 ± 0.005	1.882 ± 0.050	2.327 ± 0.047	Synergistic
SDS 99% + Cd(II) 1%(ABCR1)	0.647 ± 0.021	1.547 ± 0.051	1.910 ± 0.112	Synergistic
SDS 98% + Cd(II) 2%(ABCR2)	0.599 ± 0.018	1.671 ± 0.052	1.934 ± 0.048	Synergistic
SDS 96% + Cd(II) 4%(ABCR3)	1.805 ± 0.122	0.556 ± 0.038	0.606 ± 0.045	Antagonistic
SDS + Pb(II)				
SDS 96.07% + Pb(II) 3.93% (EECR50)	0.182 ± 0.024	5.556 ± 0.752	8.022 ± 1.253	Synergistic
SDS 97% + Pb(II) 3% (ABCR1)	0.141 ± 0.003	7.084 ± 0.156	9.725 ± 0.211	Synergistic
SDS 95% + Pb(II) 5% (ABCR2)	0.114 ± 0.004	8.796 ± 0.293	13.275 ± 0.660	Synergistic
SDS 94% + Pb(II) 6% (ABCR3)	0.178 ± 0.004	5.606 ± 0.128	8.913 ± 0.180	Synergistic
SDS + Zn(II)				
SDS 98.70% + Zn(II) 1.30% (EECR50)	0.483 ± 0,020	2.072 ± 0.088	2.904 ± 0.021	Synergistic
SDS 98% + Zn(II) 2% (ABCR1)	0.345 ± 0.021	2.796 ± 0.023	3.509 ± 0.213	Synergistic
SDS 90% + Zn(II) 10% (ABCR2)	0.543 ± 0.005	1.842 ± 0.016	2.169 ± 0.019	Synergistic
SDS 96% + Zn(II) 4% (ABCR3)	0.317 ± 0.008	3.161 ± 0.080	3.982 ± 0.080	Synergistic
SDS + Co(II)				
SDS 99.07% + Co(II) 0.93% (EECR50)	0.218 ± 0.000	2.960 ± 0.147	2.928 ± 0.467	Synergistic
SDS 99% + Co(II) 1% (ABCR1)	0.279 ± 0.012	3.595 ± 0.152	3.820 ± 0.178	Synergistic
SDS 97% + Co(II) 3% (ABCR2)	0.492 ± 0.056	2.051 ± 0.228	2.318 ± 0.117	Synergistic
SDS 93% + Co(II) 7% (ABCR3)	0.365 ± 0.054	2.780 ± 0.408	3.105 ± 0.398	Synergistic

[+]Values are reported as Mean ± 1SD

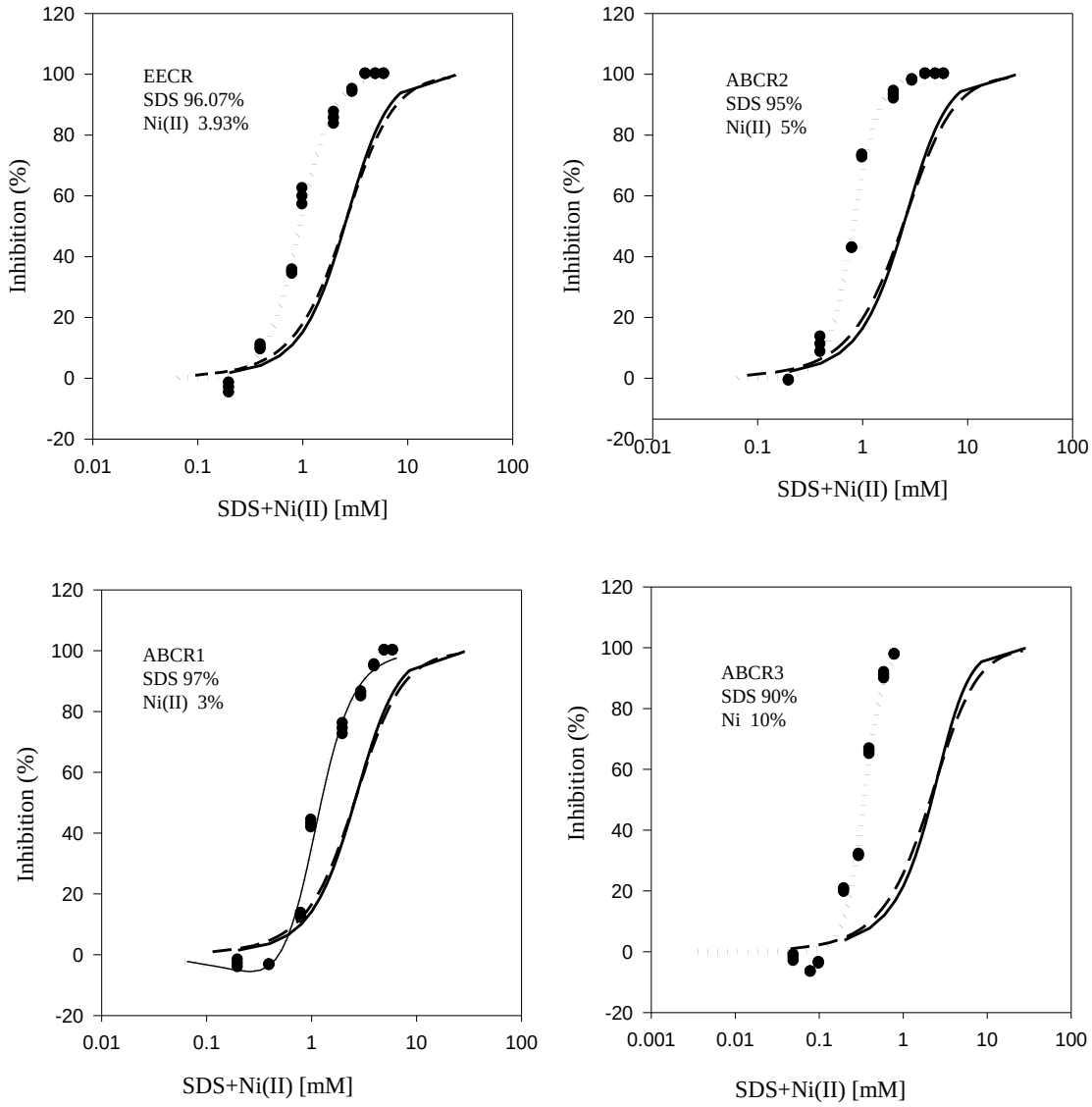


Figure 4.11: Experimental and predicted inhibitory effectsof binary mixtures ofSDS and nickel ionson *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2) or hormetic model (Eq. 3). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

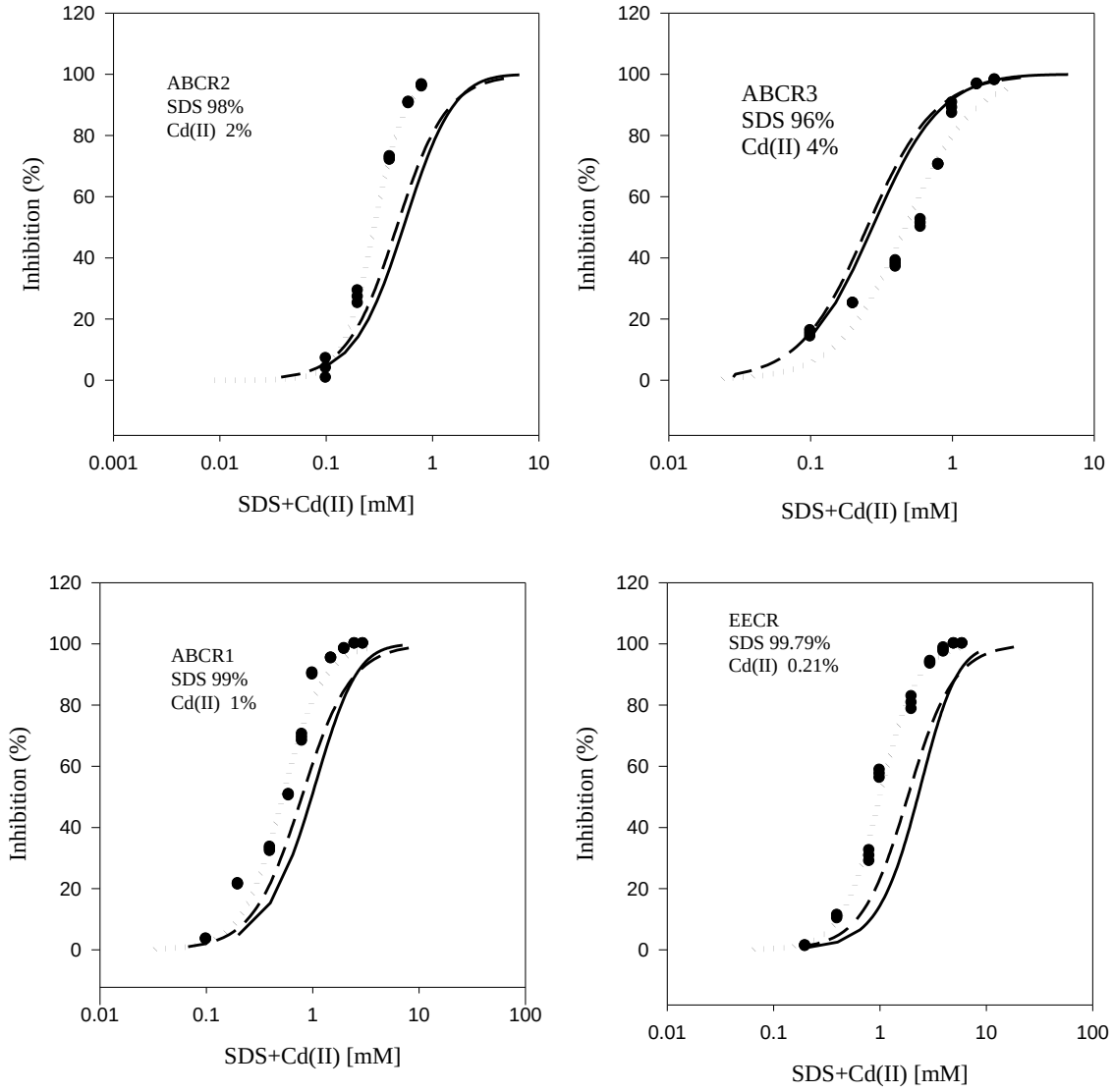


Figure 4.12: Experimental and predicted inhibitory effects of binary mixtures ofSDS and cadmium ions on *A.seifertii* dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

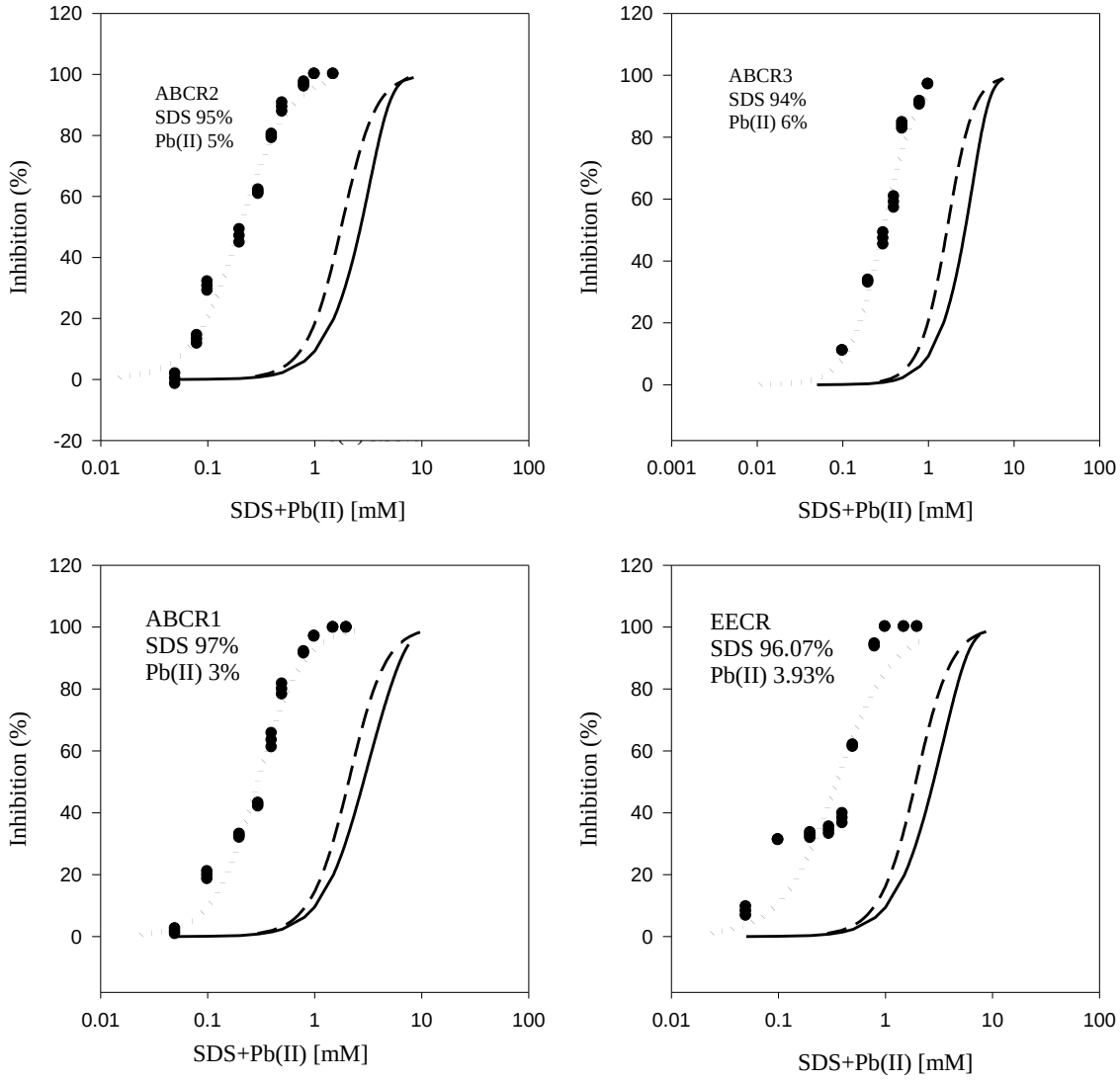


Figure 4.13: Experimental and predicted inhibitory effects of binary mixtures ofSDS and lead ion on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

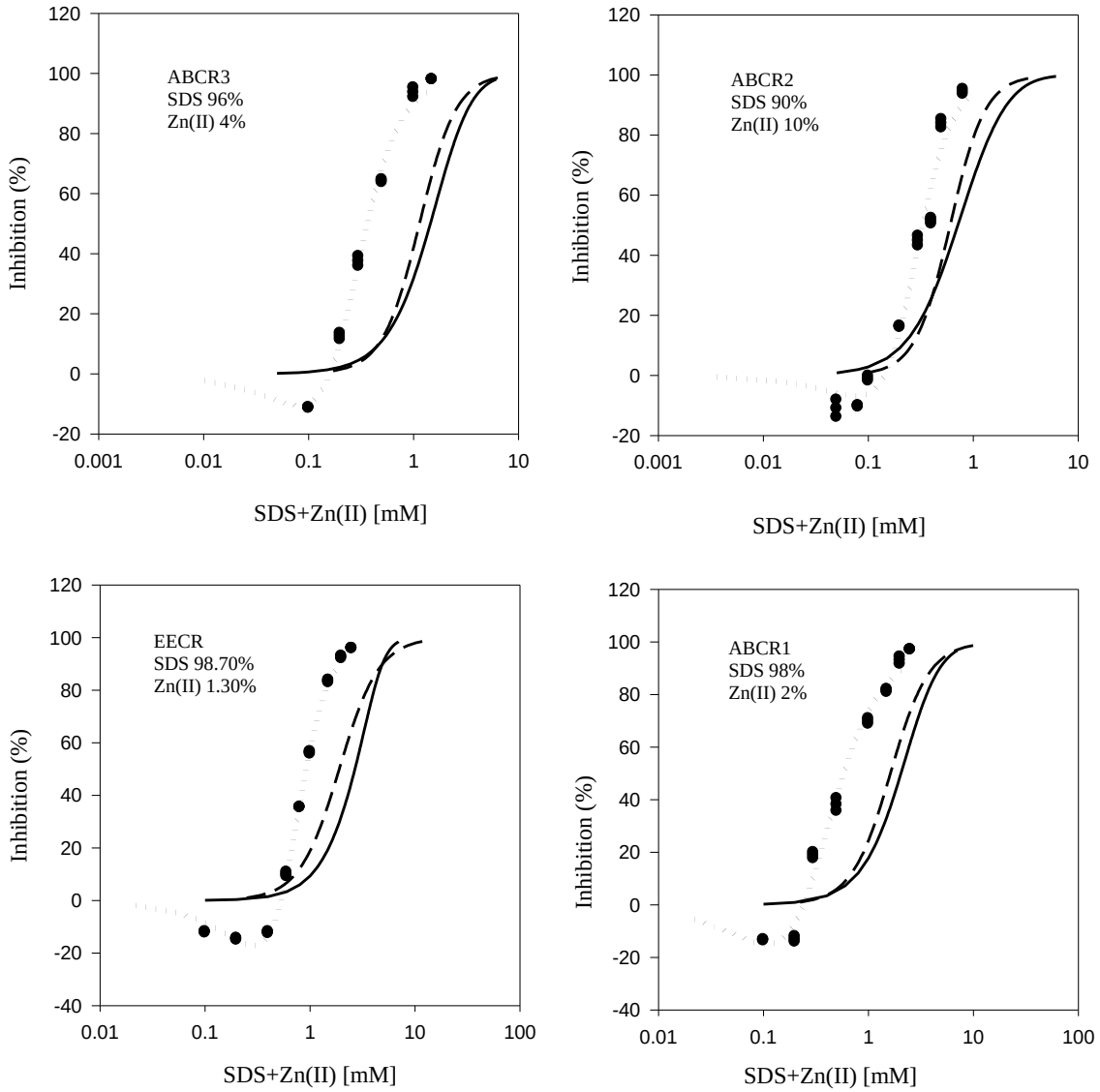


Figure 4.14: Experimental and predicted inhibitory effects of binary mixtures ofSDS and zinc ions on *A.seifertii* dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2) or hormetic model (Eq. 3). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

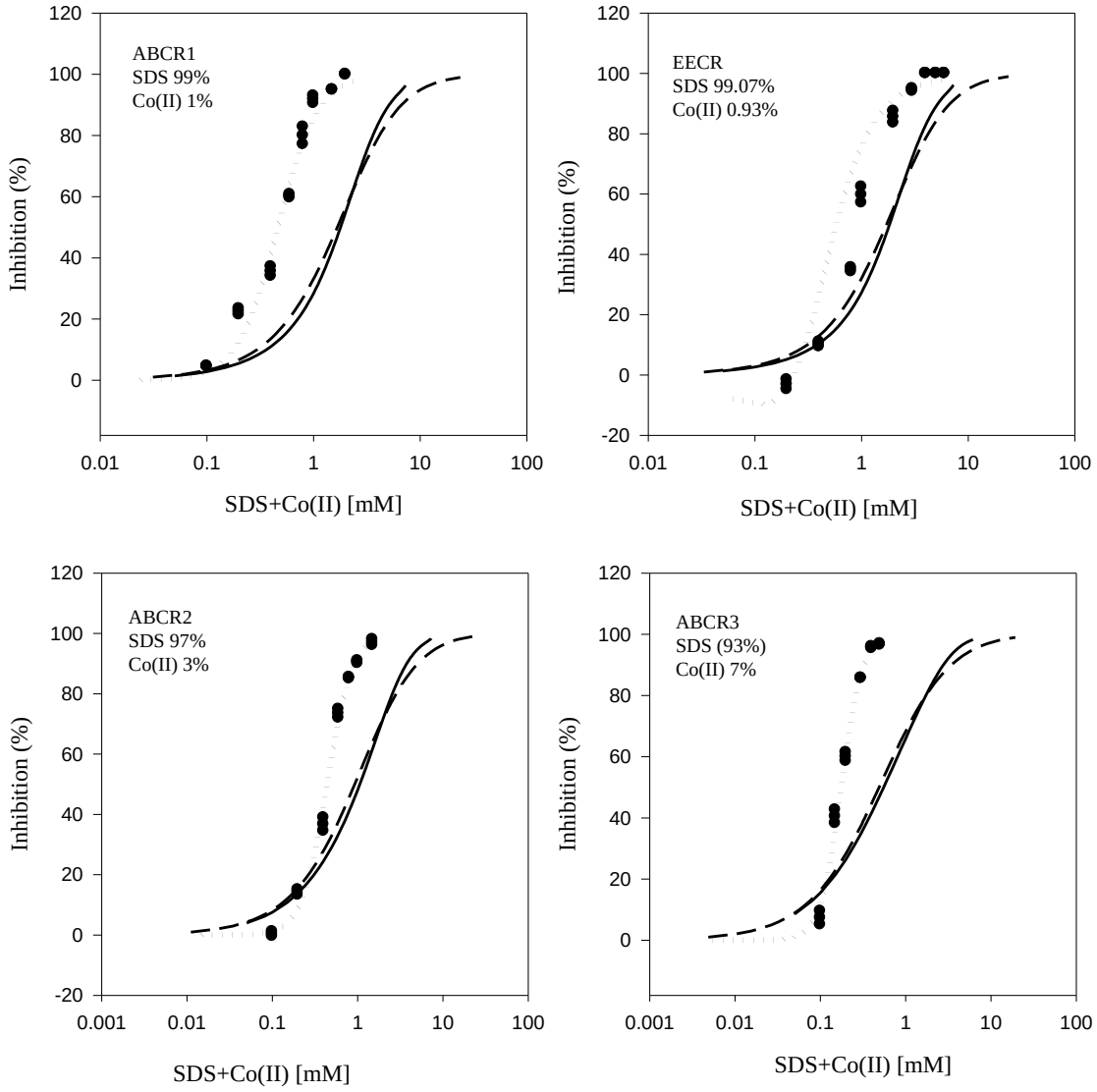


Figure 4.15: Experimental and predicted inhibitory effects of binary mixtures ofSDS and cobalt ionson *A.seifertii*dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model Eq. 2 or hormetic model (Eq. 3). Dashed and solid lines represent toxicities predicted from the concentration additionthe independent action models.

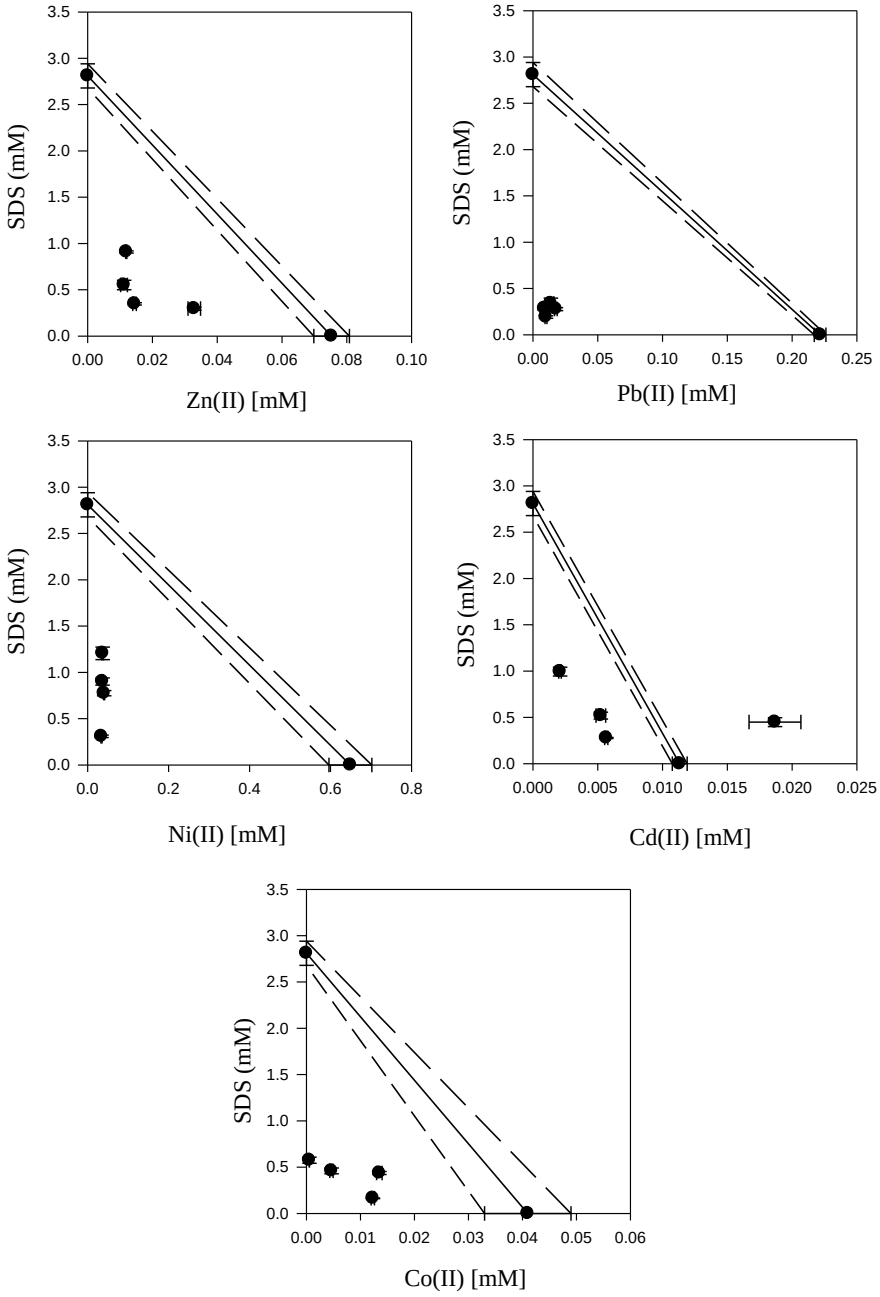


Figure4.16: The EC_{50} isobole representation for SDS and metal ions as individual and mixtures tested against dehydrogenase activity of *A.seifertii.* The thick dots represent the standard deviation of the 95% confidence interval of the values. The solid and dashed lines represent additivity line and its 95% confidence belt.

4.3.3.Toxicity of ternary mixtures

4.3.3.1.Toxicity of ternary mixtures of SDS and metals to*S. marcescens (SerEW01)*

Table 4.10 shows the experimentally-derived and predicted toxicity thresholds (EC_{50}) of ternary mixtures of metals and SDS on *S. marcescens* (SerEW01). The experimentally-derived $EC_{50S}$of SDS + Pb(II)+ Zn(II) showed that EECR50mixture ratio had the highest EC_{50}(0.181±0.010mM) while ABCR1 mixture ratio had the least (0.102±0.006mM). Similarly, among the experimentally-derived EC_{50S}, the ABCR1and ABCR2 mixture ratios were statistically different from EECR50 and ABCR3. The same trend was observed in SDS + Co(II) + Cd(II) ternary mixtures. In SDS+Cd(II)+Zn(II)ternary mixtures, the experimentally-derived EC_{50S}ranged from 0.111±0.002mM(ABCR1) to 0.203±0.009mM (EECR50). Inaddition, in both SDS + Cd(II) + Zn(II) and SDS + Pb(II) + Ni(II) ternary mixture types, the experimentally-derived EC_{50S}revealed thatEECR50mixture ratio was statistically different from the other EC_{50S}.

In SDS+Ni(II)+Cd(II) mixtures, ABCR2 mixture ratio was the most toxic (0.121 ± 0.006 mM) while ABCR1 was the least (0.202 ± 0.006 mM). Similarly, only ABCR1mixture ratio was statistically different from the others mixture ratios. In addition, in SDS + Co(II) + Pb(II) mixture type, only ABCR3mixture ratio was statistically different from the other experimentally-derived EC_{50S}.However, in all mixture ratios in the various ternay mixtures, the experimentally-derivedEC_{50S}, CA- and IA-predicted were significantly different from oneanother ($P < 0.05$).

Toxic index, model deviation ratio and effect of metals and SDS ternary mixtures on *S. marcescens* (SerEW01)are shown in Table 4.11. The toxic index (TI) values ranged from 0.086±0.023 to 0.276±0.010, while model deviation ratio (MDR) ranged from 3.642±0.134to

10.219±0.353 for CA and 5.118±0.145 to 15.853±1.281 for IA.In all mixture ratios tested, the metals and SDS ternary mixtures were synergistic in their action on the bacterium. Similarly, the experimental dose-response relationships of the ternary mixtures as well as the predictions made from CA and IA models for *S. marcescens* (SerEW01) are shown in Figures 4.17- 4.22. All the ternary mixtures of SDS and metal ions showed that both CA and IA models greatly predicted lower toxicities at all mixture ratios, compared to the experimental data and were equally toxic even at low concentrations.

Table 4.10: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Ternary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

	EC_{50} (mM)‡ [+]		
Toxicant TernaryMixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
SDS + Pb(II) + Zn(II)			
SDS 94.60% + Pb 4.16% + Zn 1.24% (EECR50)	0.181 ± 0.010a*	0.960 ± 0.048**	1.530 ± 0.030***
SDS 95% + Pb 4% + Zn 1% (ABCR1)	0.102 ± 0.006b*	1.023 ± 0.050**	1.617 ± 0.030***
SDS 93% + Pb 5% + Zn 2% (ABCR2)	0.115 ± 0.007b*	0.785 ± 0.042**	1.261 ± 0.022***
SDS 94% + Pb 2% + Zn 4% (ABCR3)	0.144 ± 0.007c*	0.692 ± 0.043**	0.987 ± 0.026***
SDS + Cd(II) +Zn(II)			
SDS 96.52% + Cd 2.21% + Zn 1.27%(EECR50)	0.203 ± 0.009a*	1.376 ± 0.073**	1.833 ± 0.008***
SDS 96% + Cd 2% + Zn 2% (ABCR1)	0.111 ± 0.020b*	1.138 ± 0.065**	1.526 ± 0.014***
SDS 94% + Cd 2% + Zn 4% (ABCR2)	0.120 ± 0.009b*	0.768 ± 0.049**	0.999 ± 0.033***
SDS 93% + Cd 3% + Zn 4% (ABCR3)	0.117 ± 0.004b*	0.761 ± 0.048**	1.000 ± 0.032***
SDS + Pb(II) +Ni(II)			
SDS 89.80% + Pb 3.95% + Ni 6.25%(EECR50)	0.203 ± 0.005a*	0.736 ± 0.045**	1.072 ± 0.006***
SDS 90% + Pb 4% + Ni 6% (ABCR1)	0.150 ± 0.006b*	0.747 ± 0.046**	1.090 ± 0.007***
SDS 88% + Pb 5% + Ni 7% (ABCR2)	0.141 ± 0.010b*	0.659 ± 0.041**	0.961 ± 0.006***
SDS 87% + Pb 2% + Ni 11% (ABCR3)	0.135 ± 0.005b*	0.697 ± 0.043**	0.803 ± 0.032***
SDS + Ni(II) + Cd(II)			
SDS 91.53% +Ni 6.37% + Cd 2.10%(EECR50)	0.130 ± 0.006a*	0.719 ± 0.042**	0.993 ± 0.004***
SDS 92% +Ni 6% + Cd 2% (ABCR1)	0.202 ± 0.006b*	0.747 ± 0.044**	1.033 ± 0.004***
SDS 90% +Ni 7% + Cd 3% (ABCR2)	0.121 ± 0.006a*	0.624 ± 0.036**	0.856 ± 0.003***
SDS 93% +Ni 5% + Cd 2% (ABCR3)	0.130 ± 0.007a*	0.806 ± 0.045**	1.116 ± 0.005***
SDS + Co(II) + Pb(II)			
SDS 94.02% + Co 1.84% + Pb 4.14%(EECR50)	0.141 ± 0.010a*	1.017 ± 0.040**	1.679 ± 0040***
SDS 94% + Co 4% + Pb 2% (ABCR1)	0.142 ± 0.007a*	0.958 ± 0.034**	1.517 ± 0.001***
SDS 93% + Co 4% + Pb 3% (ABCR2)	0.137 ± 0.008a*	0.886 ± 0.039**	1.460 ± 0.018***
SDS 95% + Co 3% + Pb 2% (ABCR3)	0.167 ± 0.008b*	1.073 ± 0.039**	1.721 ± 0.012***
SDS + Co(II) + Cd(II)			
SDS 95.93% + Co 1.88% + Cd 2.20%(EECR50)	0.165 ± 0.012a*	0.991 ± 0.035**	1.556 ± 0.007***
SDS 94% + Co 3% + Cd 3% (ABCR1)	0.135 ± 0.007b*	0.788 ± 0.027**	1.260 ± 0.011***
SDS 95% + Co 2% + Cd 3% (ABCR2)	0.143 ± 0.009b*	0.864 ± 0.030**	1.346 ± 0.005***
SDS 96% + Co 2% + Cd 2% (ABCR3)	0.118 ± 0.005c*	1.011 ± 0.036**	1.597 ± 0.009***

[+]Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other (P <0.05).

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other (P <0.05).

Table 4.11: Toxic Index, Model Deviation Ratio and Effect of Metals andSDS Ternary Mixtures on*S. marcescens* (SerEW01)

Metal+SDS Mixtures	Toxic Index (TI)$^{+}$	MDR^{+}		Effect
		CA	IA	
SDS +Pb (II)+Zn(II)				
SDS 94.60% +Pb(II) 4.16%+Zn(II) 1.24% (EECR 50)	0.188 ± 0.001	5.312 ± 0.021	8.492 ± 0.608	Synergistic
SDS 95% +Pb(II) 4%+Zn(II)1% (ABCR1)	0.086 ± 0.023	10.000±0.144	15.853±1.287	Synergistic
SDS 93% +Pb(II) 5%+Zn(II) 2% (ABCR2)	0.131 ± 0.026	6.811 ± 0.080	10.973±0.898	Synergistic
SDS 94% +Pb(II) 2%+Zn(II) 4% (ABCR3)	0.208 ± 0.003	4.807 ± 0.064	6.859 ± 0.156	Synergistic
SDS +Cd (II)+Zn(II)				
SDS 96.52% +Cd(II) 2.21%+Zn(II)1.27% (EECR50)	0.218 ± 0.005	6.766 ± 0.041	9.027 ± 0.387	Synergistic
SDS 96% +Cd(II) 2%+Zn(II) 2%(ABCR1)	0.133 ± 0.004	10.219±0.353	13.709±0.188	Synergistic
SDS 94% +Cd(II) 2%+Zn(II) 4%(ABCR2)	0.194 ± 0.010	6.422 ± 0.068	8.366 ± 0.327	Synergistic
SDS 93% +Cd(II) 3%+Zn(II) 4%(ABCR3)	0.208 ± 0.006	6.516 ± 0.218	8.574 ± 0.031	Synergistic
SDS +Pb (II)+Ni(II)				
SDS 89.80% +Pb(II) 3.95%+Ni(II) 6.25% (EECR50)	0.276 ± 0.010	3.624 ± 0.134	5.285 ± 0.149	Synergistic
SDS 90% +Pb(II) 4%+Ni(II) 6% (ABCR1)	0.201 ± 0.004	4.966 ± 0.089	7.261 ± 0.348	Synergistic
SDS 88% +Pb(II) 5%+Ni(II) 7% (ABCR2)	0.214 ± 0.002	4.673 ± 0.044	6.840 ± 0.510	Synergistic
SDS 87% +Pb(II) 2%+Ni(II) 11%($ABCR_3$)	0.223 ± 0.007	4.493 ± 0.149	5.948 ± 0.024	Synergistic
SDS +Ni (II)+Cd(II)				
SDS 91.53% +Ni(II) 6.37%+Cd(II) 2.10% (EECR50)	0.181 ± 0.002	5.516 ± 0.051	7.634 ± 0.362	Synergistic
SDS 92% +Ni(II) 6%+Cd(II) 2% (ABCR1)	0.271 ± 0.008	3.697 ± 0.106	5.118 ± 0.145	Synergistic
SDS 90% +Ni(II) 7%+Cd(II) 3% (ABCR2)	0.194 ± 0.002	5.158 ± 0.042	7.083 ± 0.343	Synergistic
SDS 93% +Ni(II) 5%+Cd(II) 2% (ABCR3)	0.161 ± 0.001	6.204 ± 0.042	8.620 ± 0.460	Synergistic
SDS +Co (II)+Pb(II)				
SDS 94.02% +Co(II) 1.84%+Pb(II) 4.14% (EECR50)	0.139 ± 0.004	7.224 ± 0.232	11.952±0.876	Synergistic
SDS 94% +Co(II) 4%+Pb(II) 2%(ABCR1)	0.149 ± 0.003	6.734 ± 0.115	10.683±0.629	Synergistic
SDS 93% +Co(II) 4%+Pb(II) 3%(ABCR2)	0.155 ± 0.004	6.461 ± 0.165	10.667±0.790	Synergistic
SDS 95% +Co(II) 3%+Pb(II) 2%(ABCR3)	0.156 ± 0.002	6.425 ± 0.074	10.323±0.563	Synergistic
SDS +Co (II)+Cd(II)				
SDS 95.93% +Co(II) 1.88%+Cd(II) 2.20% (EECR50)	0.166 ± 0.006	6.029 ± 0.211	9.482 ± 0.704	Synergistic
SDS 94% +Co(II) 3%+Cd(II) 3%(ABCR1)	0.172 ± 0.004	5.844 ± 0.102	9.350 ± 0.563	Synergistic
SDS 95% +Co(II) 2%+Cd(II) 3%(ABCR2)	0.165 ± 0.005	6.052 ± 0.169	9.439 ± 0.626	Synergistic
SDS 96% +Co(II) 2%+Cd(II) 2%(ABCR3)	0.116 ± 0.001	8.597 ± 0.039	13.585±0.589	Synergistic

$^{+}$Values are reported as Mean ± 1SD

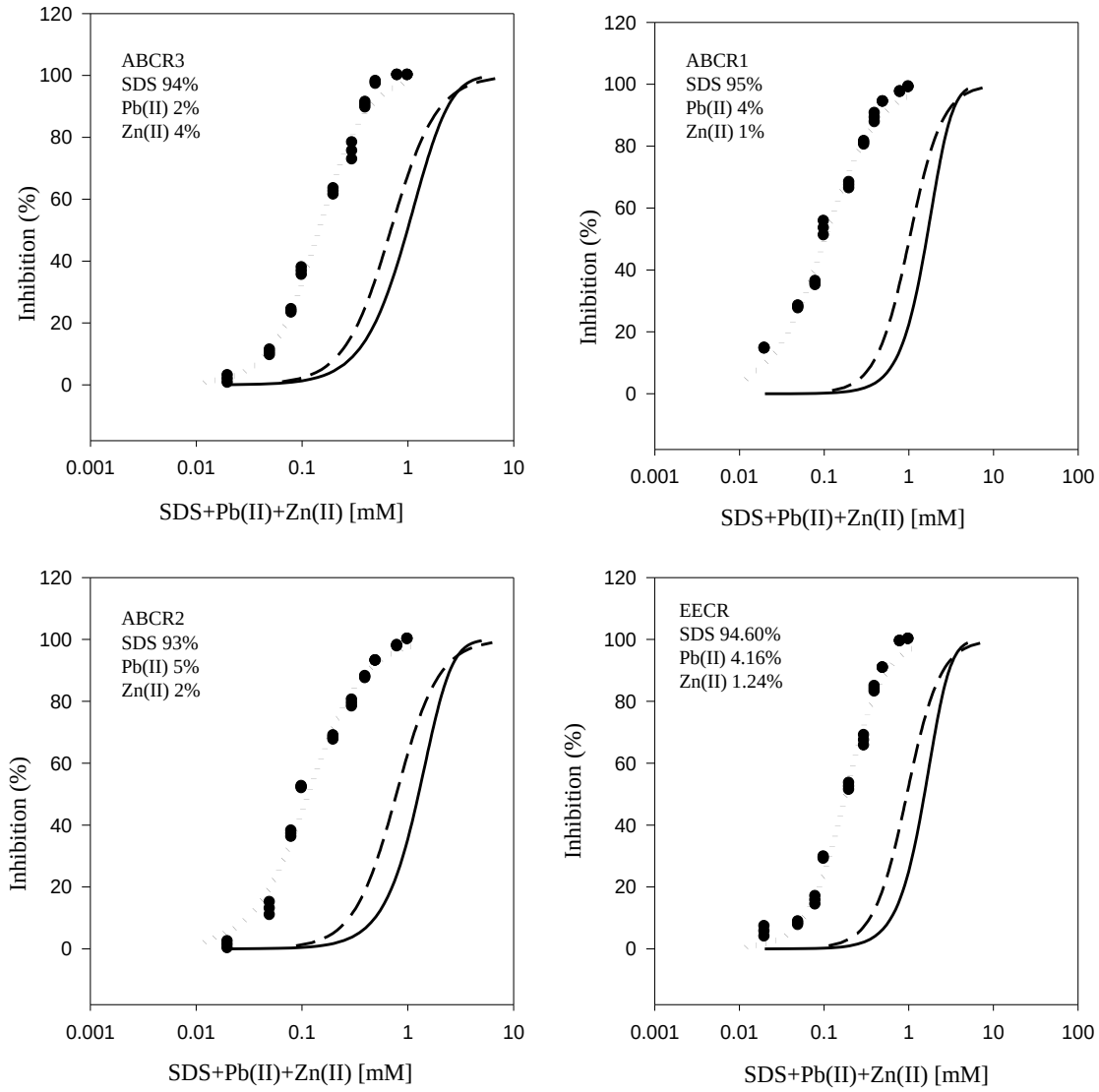


Figure 4.17: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, lead and zinc ionson *S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

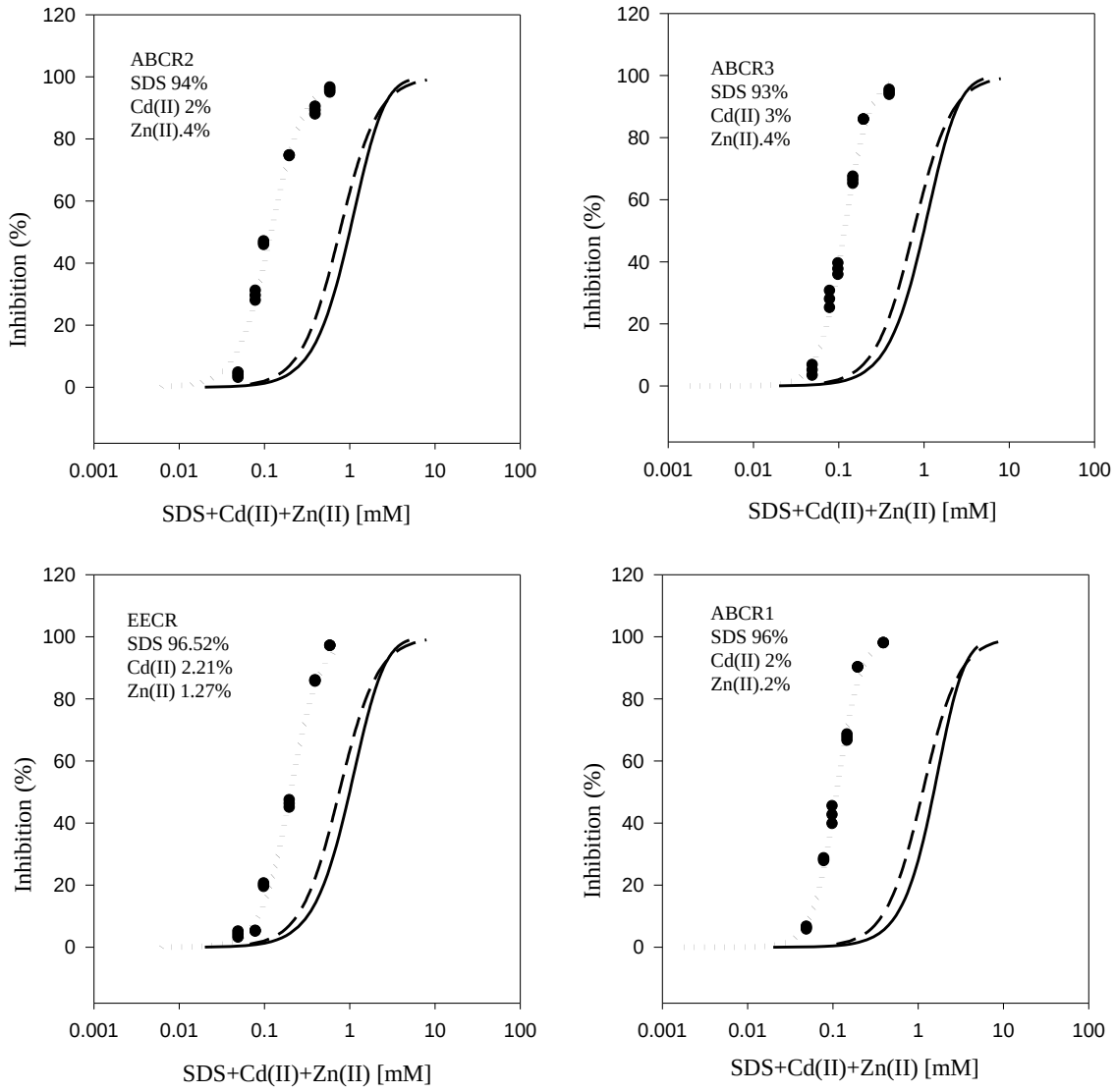


Figure 4.18: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, cadmium and zinc ions on*S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

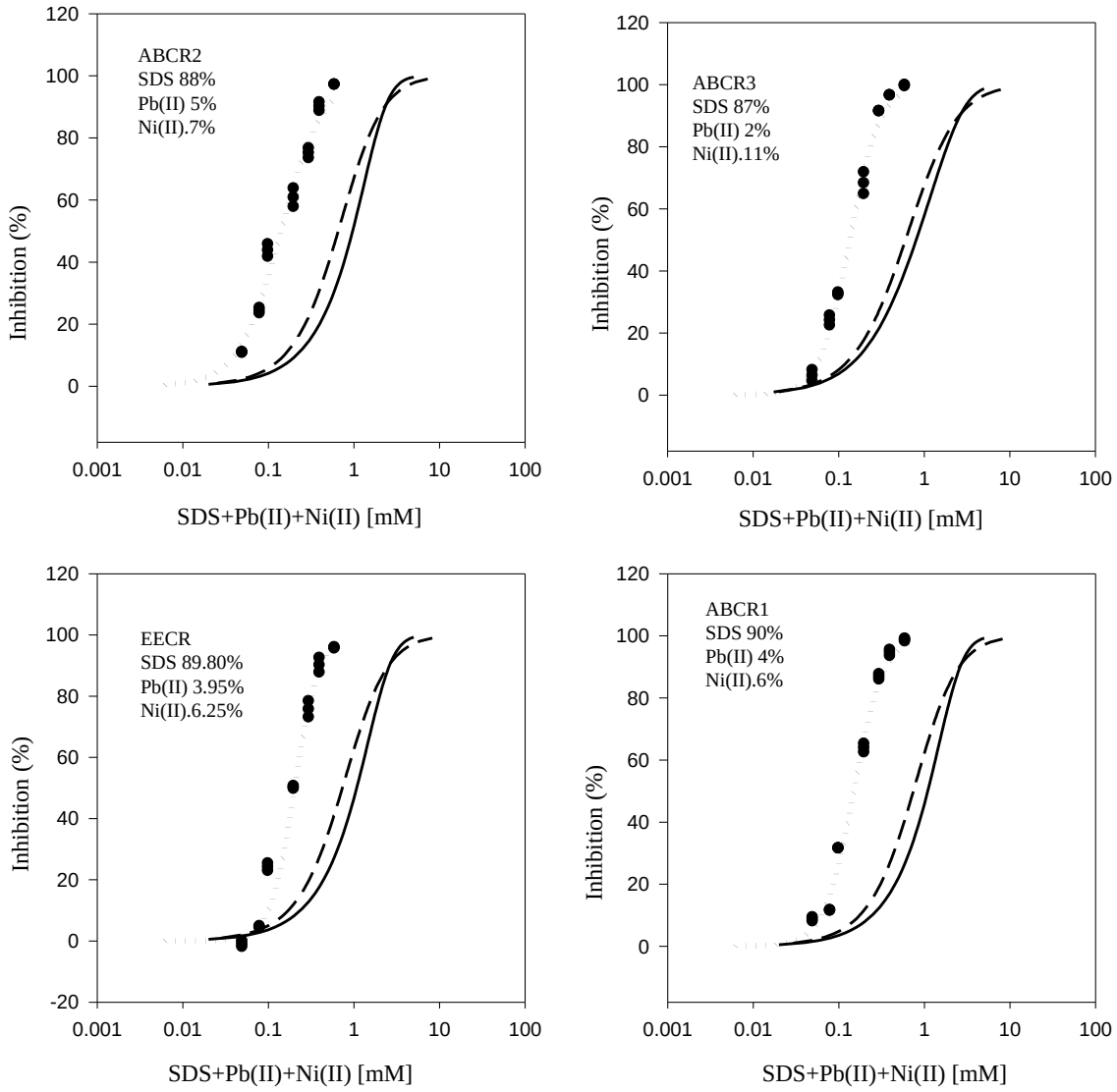


Figure 4.19: Experimental and predicted inhibitory effectsof ternary mixtures ofSDS, lead and nickel ions on *S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

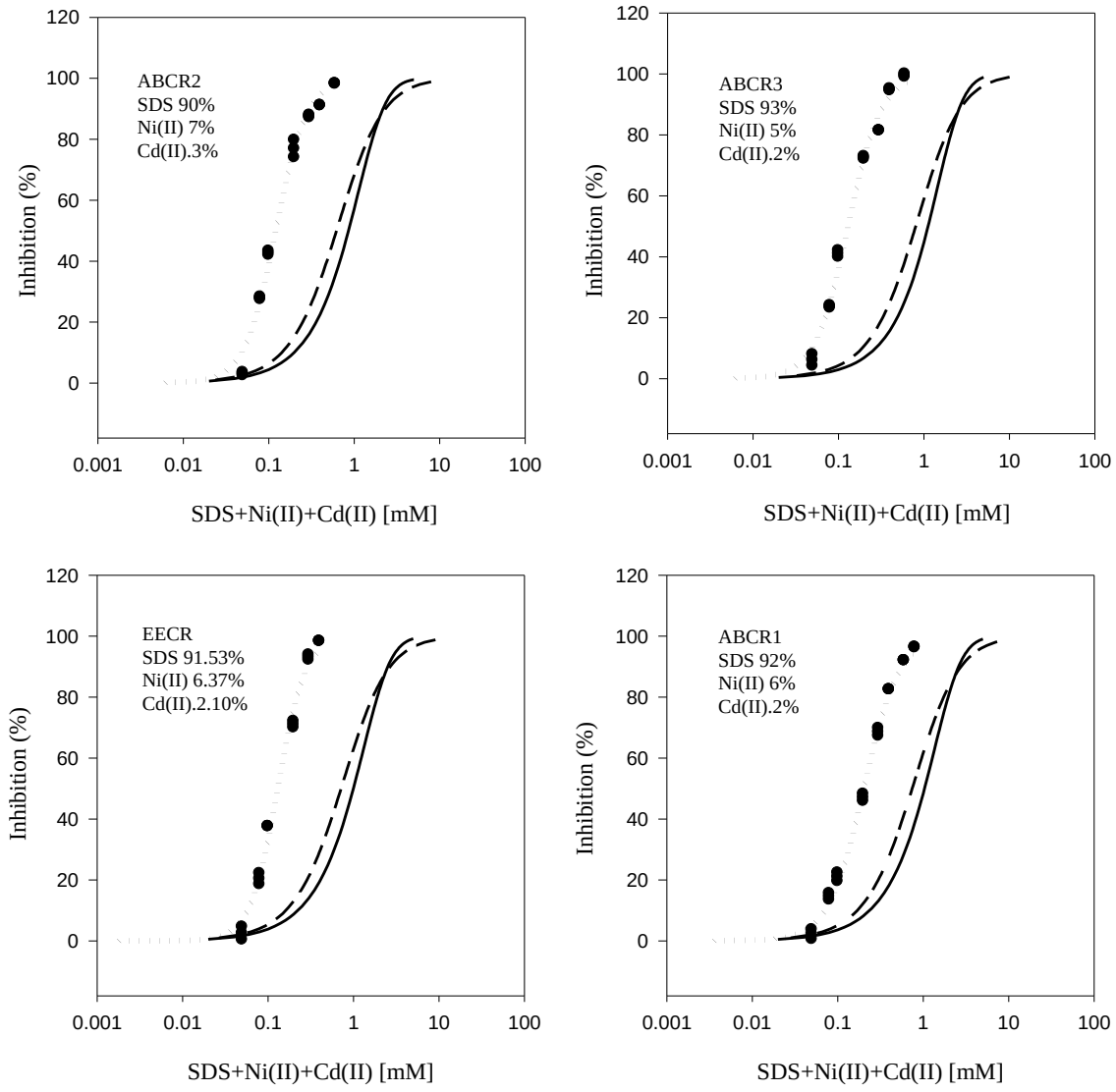


Figure 4.20: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, nickel and cadmium ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

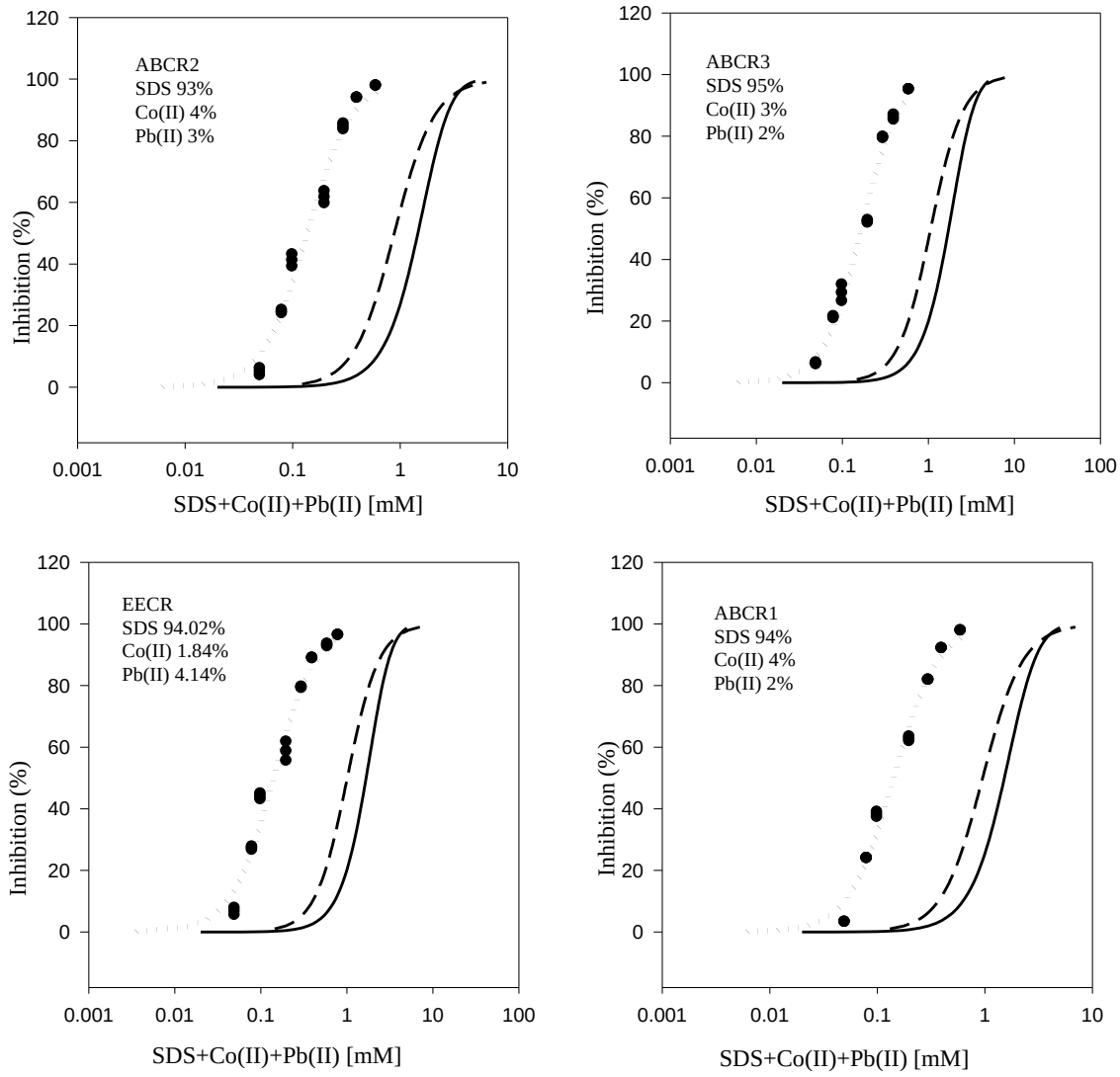


Figure 4.21: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, cobalt and lead ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

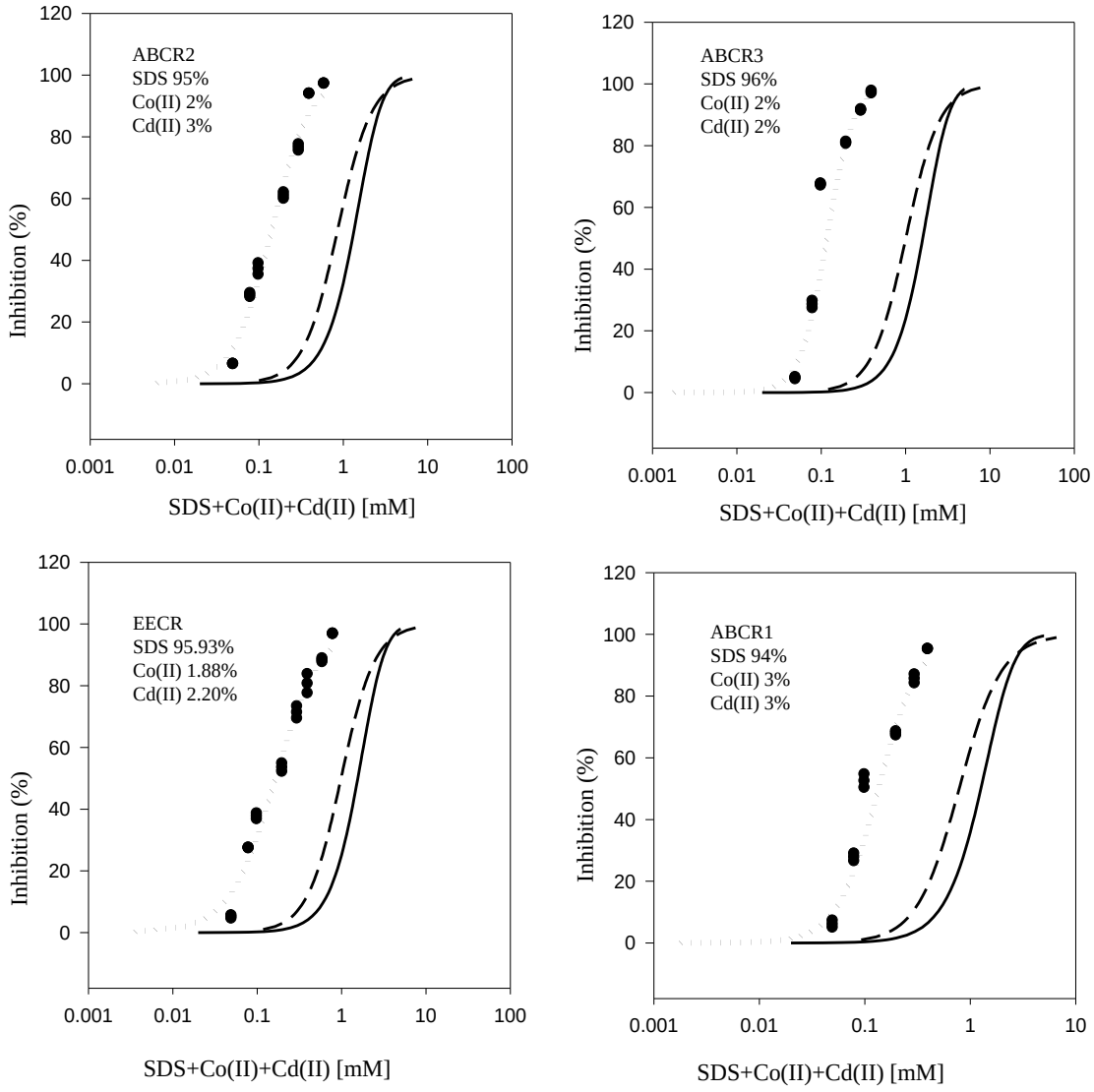


Figure 4.22: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, cobalt and cadmium ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.3.2.Toxicity of ternary mixtures of SDS and metals to*A. seifertii*

Table 4.12shows the experimentally-derived and predicted toxicity thresholds (EC_{50}) of ternary mixtures of metals and SDS on *A.seifertii*.The experimentally-derived EC_{50S}in SDS + Pb(II) +Zn(II)mixture showed that ABCR2mixture ratio was the least toxic (0.368±0.008mM) while ABCR1 mixture ratio was the most toxic (0.302±0.016mM). In addition, the EECR50and ABCR1 mixture ratios were statistically different from ABCR2 and ABCR3. In SDS+Cd(II)+Zn(II) mixtures, the experimentally-derived EC_{50} values ranged from 0.242±0.020mM (ABCR1) to 0.713±0.028mM (EECR50). The experimentally-derived EC_{50S} showed that EECR50and ABCR2 mixture ratios were statistically different from ABCR1 and ABCR3mixture ratios.In SDS+Pb(II)+Ni(II) mixtures, for the experimentally-derived EC_{50S},EECR50 and ABCR3 mixture ratios were statistically different from ABCR1 and ABCR2mixture ratios. SDS+Ni(II)+Cd(II) mixtures, the experimentally-derived EC_{50S}showed significantly difference amongthe mixture ratios. In SDS+Co(II)+Pb(II) mixtures, ABCR2 mixture ratio was the most toxic (0.197 ± 0.017 mM), while ABCR1 mixture ratio was the least (0.334 ± 0.016 mM). In SDS+Co(II)+Cd(II) mixtures, the experimentally-derived EC_{50S}showed thatthe ABCR2 and ABCR3 mixture ratios were statistically different from each other. Inaddition, in ABCR3 mixture ratio of the mixture type, there was no statistical difference between CA- and IA-predicted EC_{50S} ($P < 0.05$). Similarly, in SDS + Ni(II) + Cd(II) mixture type, the EC_{50} derived from the independent action model was statistically different from both the experimentally-derived $EC_{50}$and that predicted on the basis ofconcentration addition model of ABCR1 mixture ratio ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS ternary mixtures on*A.seifertii*are shown in Table 4.13. From the results, the toxic index (TI) values ranged from

0.139±0.003 to 0.919±0.019, while model deviation ratio (MDR) ranged from 1.088±0.023 to 7.173±0.148 for CA and 1.233±0.041 to 9.621±0.090 for IA.At all the tested mixture ratios, the ternary mixtures were synergistic in their actions on the bacterium, except for ABCR1 mixture ratio of SDS+Ni(II)+Cd(II) mixture, whose effect was rather additive.

The experimental dose-response relationships of the ternary mixtures as well as the predictions made from CA and IA models for *A.seifertii* are shown in Figures 4.23-4.28. In the SDS+Pb(II)+Zn(II) mixture as shown in Figure 4.23, both models greatly underestimated the toxicities except for ABCR3, where they slightly underestimated the toxicity.

In SDS+Cd(II) +Zn(II) and SDS+Pb(II)+Ni(II) mixtures, both CA and IA models also underestimated the toxicities relative to the experimental data as shown in Figures 4.24 and 4.25 respectively. In SDS+Ni(II)+Cd(II) mixture, the models slightly underestimated the toxicities and were toxic even at low concentrations, except in ABCR1 mixture ratio, where both models almost correctly predicted the experimentally derived data at low concentration, while slightly underestimated the mixture toxicity at high concentration. Similarly, in both SDS + Ni(II) + Cd(II) and SDS +Co(II) + Cd(II) mixtures,CAand IA models predicted similar toxicities, as their dose-response curves were almost superimposed (Figure 4.26 and 4.28). In ABCR1 mixture ratio of SDS+Co(II)+Pb(ll) and all SDS+Co(II)+Cd(II) mixtures, both models slightly predicted lower toxicities than the experimentally derived data and were toxic even at low concentrations. In other SDS + Co(II) + Pb(ll) mixture ratios, both CA and IA models however grossly under estimated the mixture toxicities as seen shown Figures 4.27 and 4.28.

Table 4.12: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Ternary Mixtures of Metals and SDS on *A.seifertii*

Toxicant Ternary Mixtures	EC_{50}(mM)‡ [+]		
	Experimental†	CA-Predicted	IA- Predicted
SDS + Pb(II) + Zn(II)			
SDS 94.87% + Pb(II) 3.88% + Zn(II) 1.25%(EECR50)	0.328 ± 0.018a*	1.473 ± 0.068**	2.384 ± 1.018***
SDS 95% + Pb(II) 4% + Zn(II) 1% (ABCR1)	0.302 ± 0.016a*	1.535 ± 0.069**	2.490 ± 0.006***
SDS 93% + Pb(II) 5% + Zn(II) 2% (ABCR2)	0.368 ± 0.008b*	1.217 ± 0.058**	2.004 ± 0.197***
SDS 90% + Pb(II) 2% + Zn(II) 8% (ABCR3)	0.349 ± 0.023b*	0.679 ± 0.044**	0.868 ± 0.927***
SDS + Cd(II) +Zn(II)			
SDS 98.50% + Cd(II) 0.20% + Zn(II) 1.30%(EECR50)	0.713 ± 0.028a*	1.630 ± 0.082**	2.335 ± 0.831***
SDS 96% + Cd(II) 1% + Zn(II) 3% (ABCR1)	0.242 ± 0.020b*	1.274 ± 0.075**	1.707 ± 0.007***
SDS 98% + Cd(II) 1% + Zn(II) 1% (ABCR2)	0.639 ± 0.023c*	1.899 ± 0.097**	2.491 ± 0.093***
SDS 95% + Cd(II) 2% + Zn(II) 3% (ABCR3)	0.270 ± 0.030b*	1.210 ± 0.068**	1.715 ± 0.005***
SDS + Pb(II) +Ni(II)			
SDS 92.44% + Pb(II) 3.78% + Ni(II) 3.78%(EECR50)	0.538 ± 0.017a*	1.793 ± 0.076**	2.527 ± 0.467***
SDS 93% + Pb(II) 3% + Ni(II) 4% (ABCR1)	0.443 ± 0.018b*	1.894 ± 0.083**	2.532 ± 0.006***
SDS 94% + Pb(II) 3% + Ni(II) 3% (ABCR2)	0.270 ± 0.006b*	1.937 ± 0.083**	2.597 ± 0.047***
SDS 91% + Pb(II) 4% + Ni(II) 5% (ABCR3)	0.421 ± 0.012a*	1.720 ± 0.074**	2.448 ± 0.057***
SDS + Ni(II) + Cd(II)			
SDS 94.21% +Ni(II) 3.86% + Cd(II) 1.93%(EECR50)	0.116 ± 0.004a*	0.477 ± 0.024**	0.544 ± 1.100***
SDS 93% +Ni(II) 5% + Cd(II) 2% (ABCR1)	0.423 ±0.018b*	0.460 ± 0.023*	0.521 ± 0.019**
SDS 94% +Ni(II) 4% + Cd(II) 2% (ABCR2)	0.267 ± 0.005c*	0.463 ± 0.023**	0.526 ± 0.023***
SDS 91% +Ni(II) 6% + Cd(II) 3% (ABCR3)	0.184 ± 0.012d*	0.326 ± 0.017**	0.357 ± 0.103***
SDS + Co(II) + Pb(II)			
SDS 95.22% + Co(II)0.89% + Pb(II) 3.89%(EECR50)	0.277 ± 0.005a*	1.362 ± 0.117**	1.873 ± 0.879***
SDS 94% + Co(II) 3% + Pb(II) 3% (ABCR1)	0.334 ± 0.016b*	0.829 ± 0.112**	1.056 ± 0.045***
SDS 95% + Co(II) 3% + Pb(II) 2% (ABCR2)	0.197 ± 0.017c*	0.859 ± 0.120**	1.052 ± 0.455***
SDS 96% + Co(II) 2% + Pb(II) 2% (ABCR3)	0.261 ± 0.008a*	1.093 ± 0.134**	1.333 ± 0.138***
SDS + Co(II) + Cd(II)			
SDS 97.10% + Co(II) 0.91% + Cd(II).2%(EECR50)	0.198 ± 0.016a*	0.428 ± 0.027**	0.480 ± 0.530***
SDS 98% + Co(II) 1% + Cd(II) 1% (ABCR1)	0.216 ± 0.008a*	0.676 ± 0.049**	0.801 ± 0.001***
SDS 96% + Co(II) 2% + Cd(II) 2% (ABCR2)	0.248 ± 0.011b*	0.385 ± 0.029**	0.425 ± 0.035***
SDS 95% + Co(II) 3% + Cd(II) 2% (ABCR3)	0.169 ± 0.008c*	0.352 ± 0.030**	0.388 ± 0.235**

[+]Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other (P <0.05).

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other (P <0.05).

Table 4.13: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Ternary Mixtures on*A.seifertii*

	MDR[+]

Metal-SDS Mixtures	Toxic Index (TI) [+]	CA	IA	Effect
SDS +Pb (II)+Zn(II)				
SDS 94.87% +Pb(II) 3.88%+Zn(II) 1.25% (EECR 50)	0.223 ± 0.002	4.493 ± 0.039	7.282 ± 0.384	Synergistic
SDS 95% +Pb(II) 4%+Zn(II)1% (ABCR1)	0.196 ± 0.001	5.090 ± 0.035	8.267 ± 0.395	Synergistic
SDS 93% +Pb(II) 5%+Zn(II) 2% (ABCR2)	0.303 ± 0.008	3.304 ± 0.087	5.558 ± 0.136	Synergistic
SDS 90% +Pb(II) 2%+Zn(II) 8% (ABCR3)	0.514 ± 0.002	1.946 ± 0.006	2.491 ± 0.553	Synergistic
SDS +Cd (II)+Zn(II)				
SDS 98.50% +Cd(II) 0.20%+Zn(II)1.30% (EECR50)	0.499 ± 0.007	2.286 ± 0.025	3.278 ± 0.104	Synergistic
SDS 96% +Cd(II) 1%+Zn(II)3% (ABCR1)	0.393 ± 0.014	5.264 ± 0.139	7.074 ± 0.526	Synergistic
SDS 98% +Cd(II) 1%+Zn(II) 1% (ABCR2)	0.365 ± 0.006	2.969 ± 0.042	3.898 ± 0.097	Synergistic
SDS 95% +Cd(II) 2%+Zn(II) 3% (ABCR3)	0.246 ± 0.013	4.503 ± 0.243	6.406 ± 0.639	Synergistic
SDS +Pb (II)+Ni(II)				
SDS 92.44% +Pb(II) 3.78%+Ni(II) 3.78% (EECR50)	0.300 ± 0.003	3.329 ± 0.033	4.696 ± 0.122	Synergistic
SDS 93% +Pb(II) 3%+Ni(II) 4% (ABCR1)	0.234 ± 0.001	4.277 ± 0.019	5.724 ± 0.168	Synergistic
SDS 94% +Pb(II) 3%+Ni(II) 3% (ABCR2)	0.139 ± 0.003	7.173 ± 0.148	9.621 ± 0.090	Synergistic
SDS 91% +Pb(II) 4%+Ni(II) 5% (ABCR3)	0.245 ± 0.004	4.084 ± 0.060	5.818 ± 0.152	Synergistic
SDS +Ni (II)+Cd(II)				
SDS 94.21% +Ni(II) 3.86%+Cd(II) 1.93% (EECR50)	0.243 ± 0.007	4.120 ± 0.113	4.702 ± 0.122	Synergistic
SDS 93% +Ni(II) 5%+Cd(II) 2% (ABCR1)	0.919 ± 0.019	1.088 ± 0.023	1.233 ± 0.041	Additivity
SDS 94% +Ni(II) 4%+Cd(II) 2% (ABCR2)	0.578 ± 0.019	1.733 ± 0.058	1.970 ± 0.039	Synergistic
SDS 91% +Ni(II) 6%+Cd(II) 3% (ABCR3)	0.563 ± 0.017	1.779 ±0.054	1.945 ± 0.090	Synergistic
SDS +Co (II)+Pb(II)				
SDS 95.22% +Co(II) 0.89%+Pb(II) 3.89% (EECR50)	0.204 ± 0.014	4.914 ± 0.332	6.761 ± 0.079	Synergistic
SDS 94% +Co(II) 3%+Pb(II) 3% (ABCR1)	0.406 ± 0.036	2.475 ± 0.218	3.157 ± 0.137	Synergistic
SDS 95% +Co(II) 3%+Pb(II) 2% (ABCR2)	0.231 ± 0.013	4.346 ± 0.235	5.340 ± 0.084	Synergistic
SDS 96% +Co(II) 2%+Pb(II) 2% (ABCR3)	0.243 ± 0.023	4.136 ± 0.380	5.097 ± 0.143	Synergistic
SDS +Co (II)+Cd(II)				
SDS 97.10% +Co(II) 0.91%+Cd(II) 2% (EECR50)	0.463 ± 0.014	2.162 ± 0.066	2.430 ± 0.210	Synergistic
SDS 98% +Co(II) 1%+Cd(II) 1% (ABCR1)	0.320 ± 0.011	3.124 ± 0.104	3.708 ± 0.195	Synergistic
SDS 96% +Co(II) 2%+Cd(II) 2% (ABCR2)	0.647 ± 0.020	1.547 ± 0.048	1.715 ± 0.086	Synergistic
SDS 95% +Co(II) 3%+Cd(II) 2% (ABCR3)	0.483 ± 0.018	2.071 ± 0.078	2.291 ± 0.113	Synergistic

[+]Values are reported as Mean ± 1SD

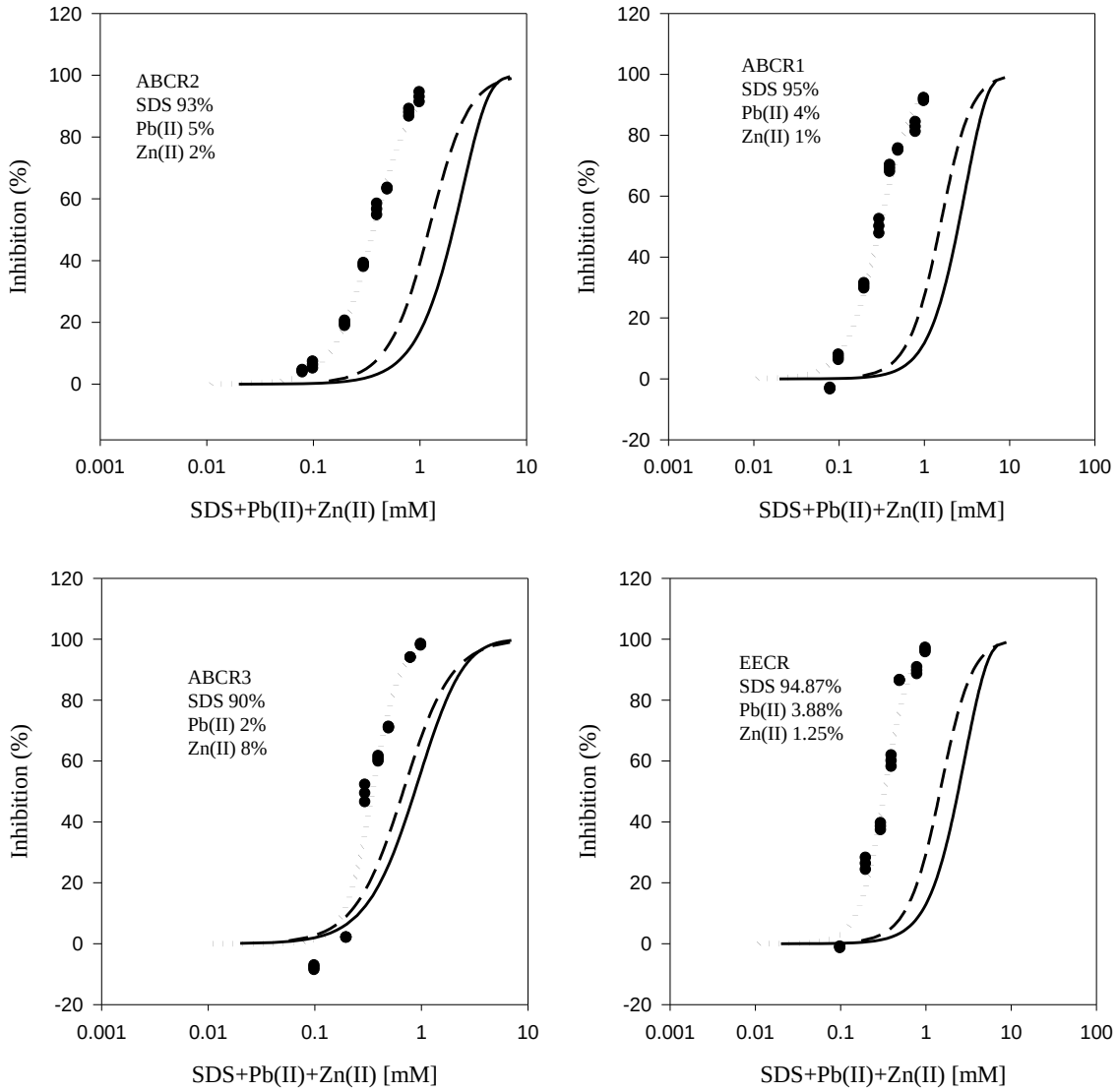


Figure 4.23: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, lead and zinc ions on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

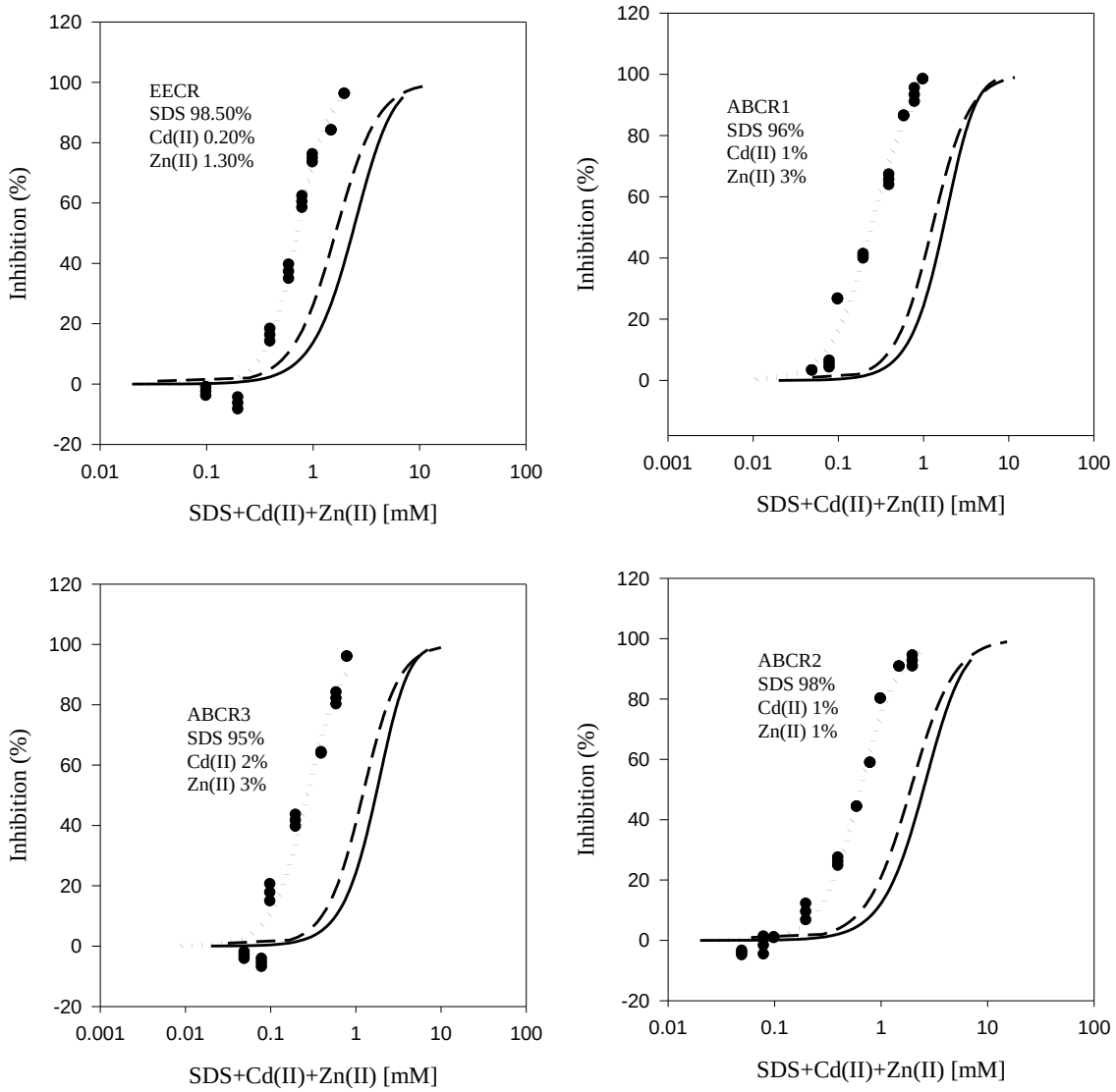


Figure 4.24: Experimental and predicted inhibitiory effectsof ternary mixtures ofSDS, cadmium and zinc ionson *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

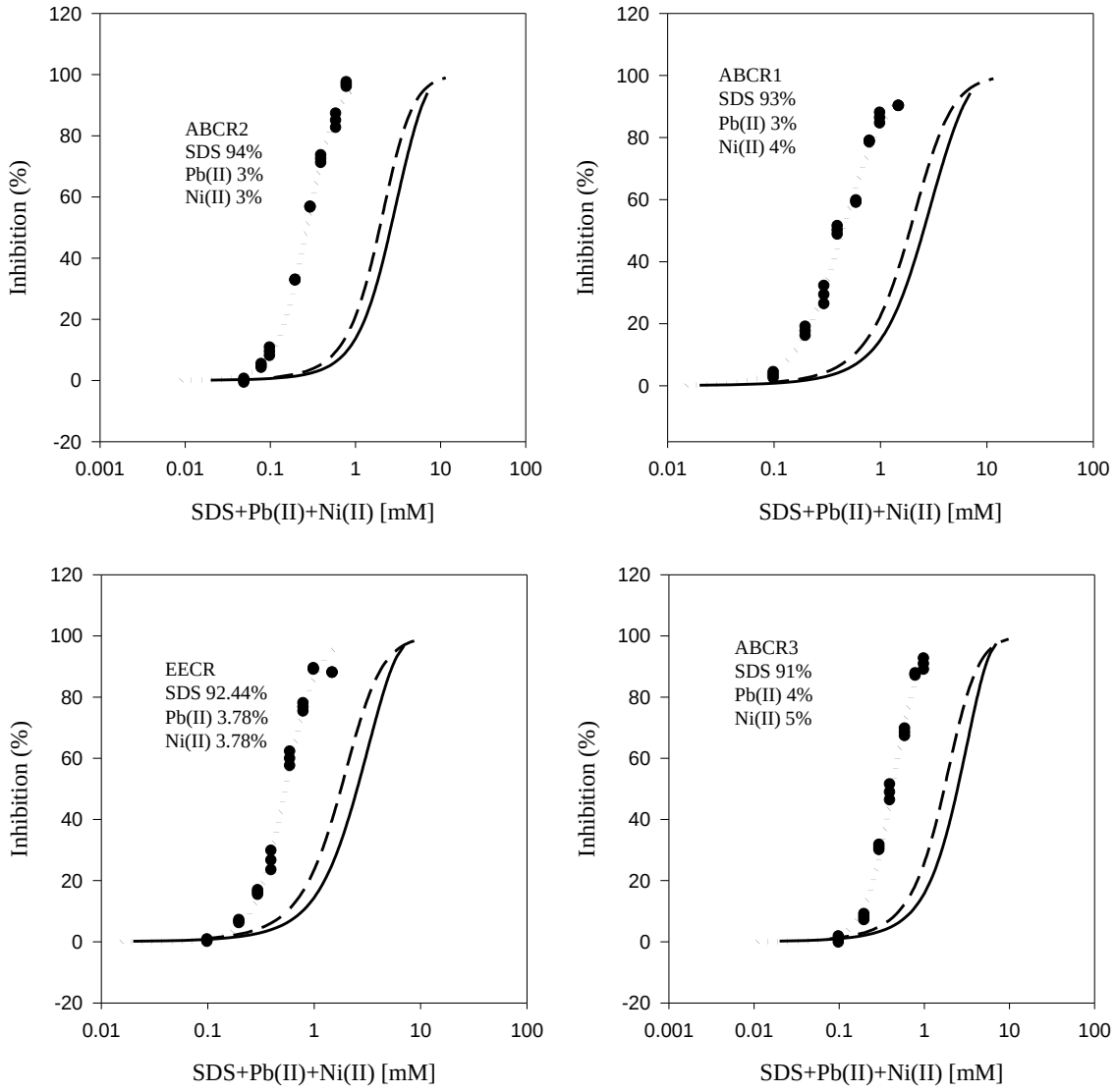


Figure 4.25: Experimental and predicted inhibitory effectsof ternary mixtures ofSDS, lead and nickel ions on *A. seifertii*dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

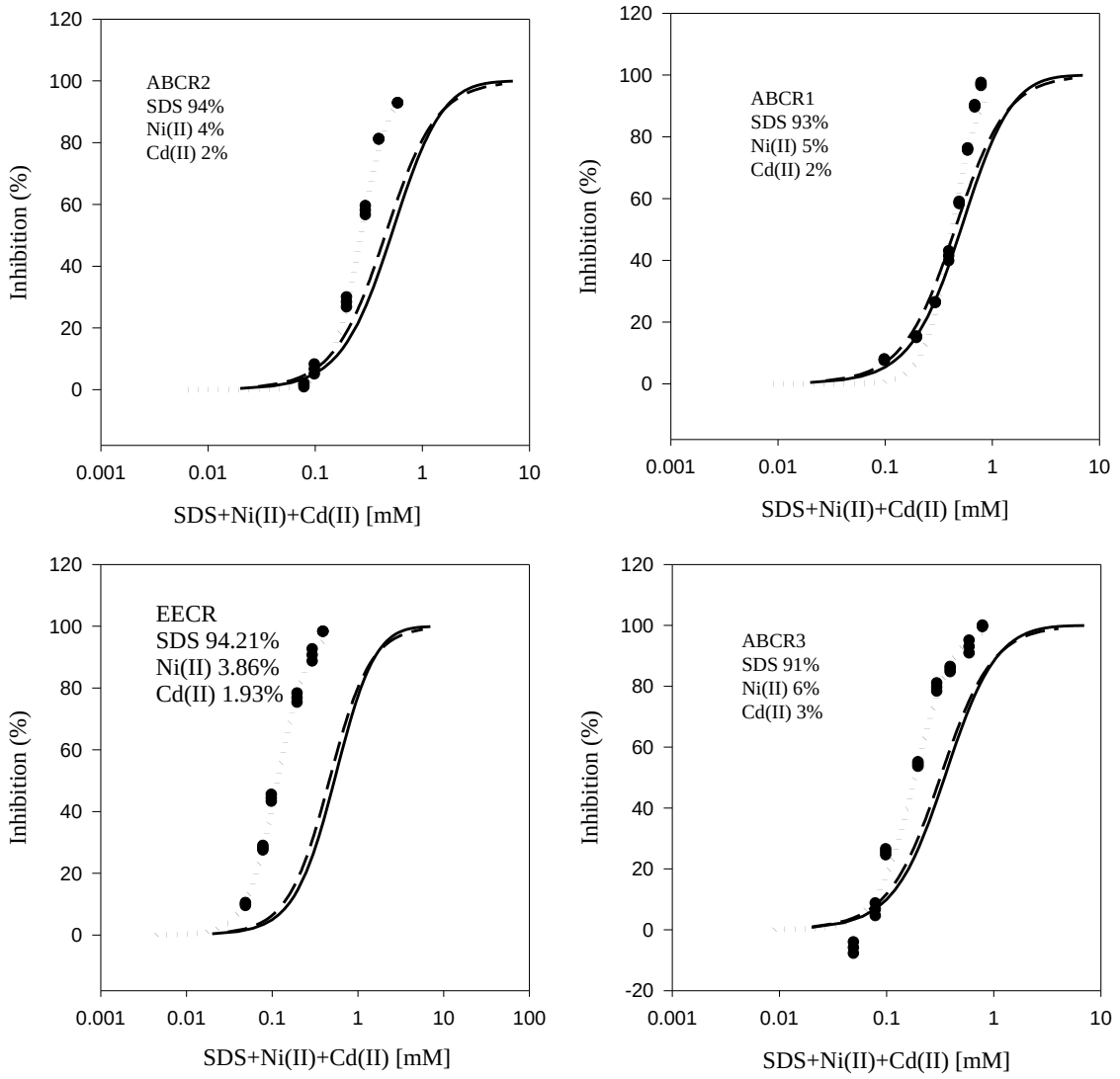


Figure 4.26: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, nickel and cadmium ions on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

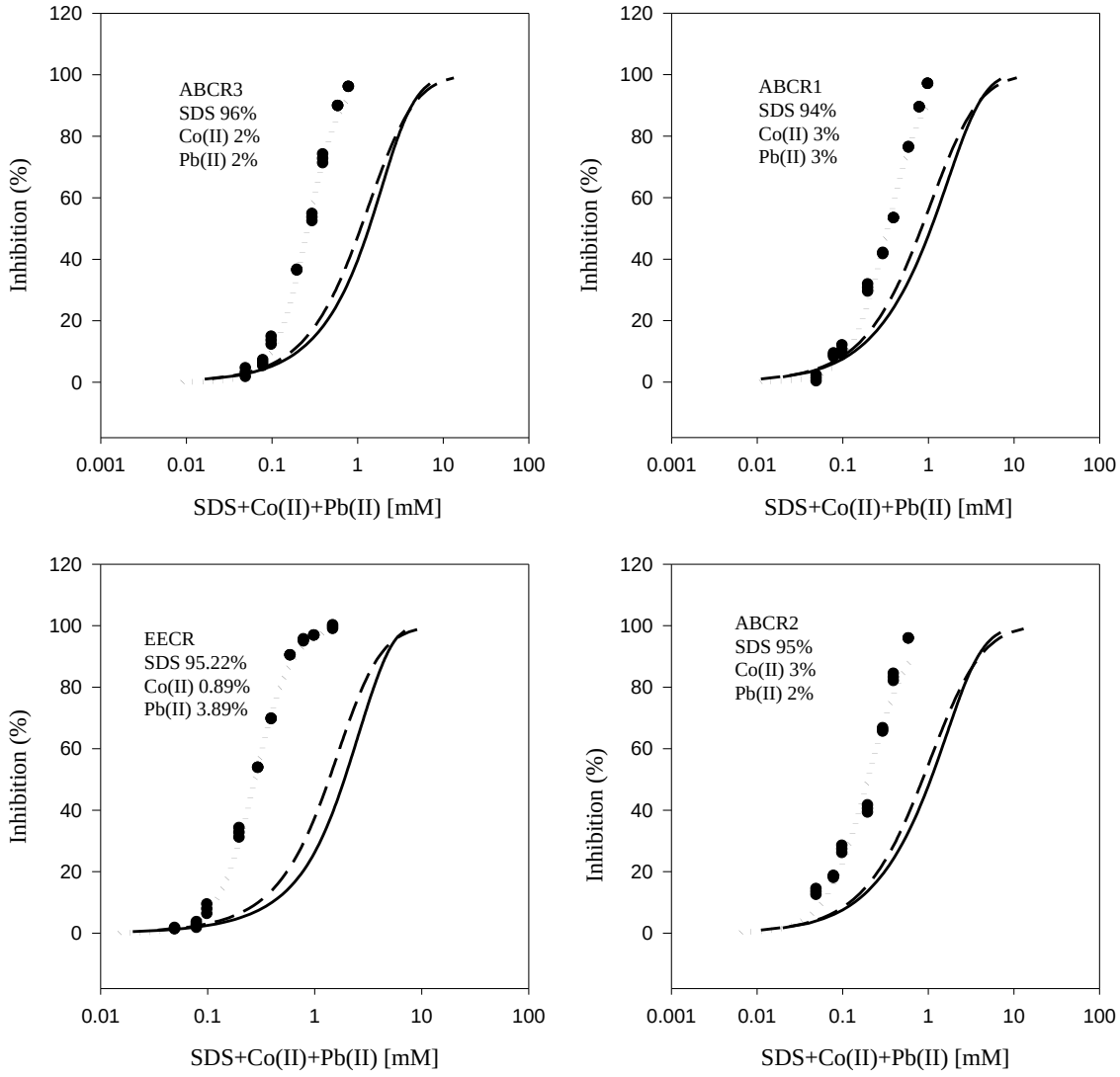


Figure 4.27: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, cobalt and lead ions on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

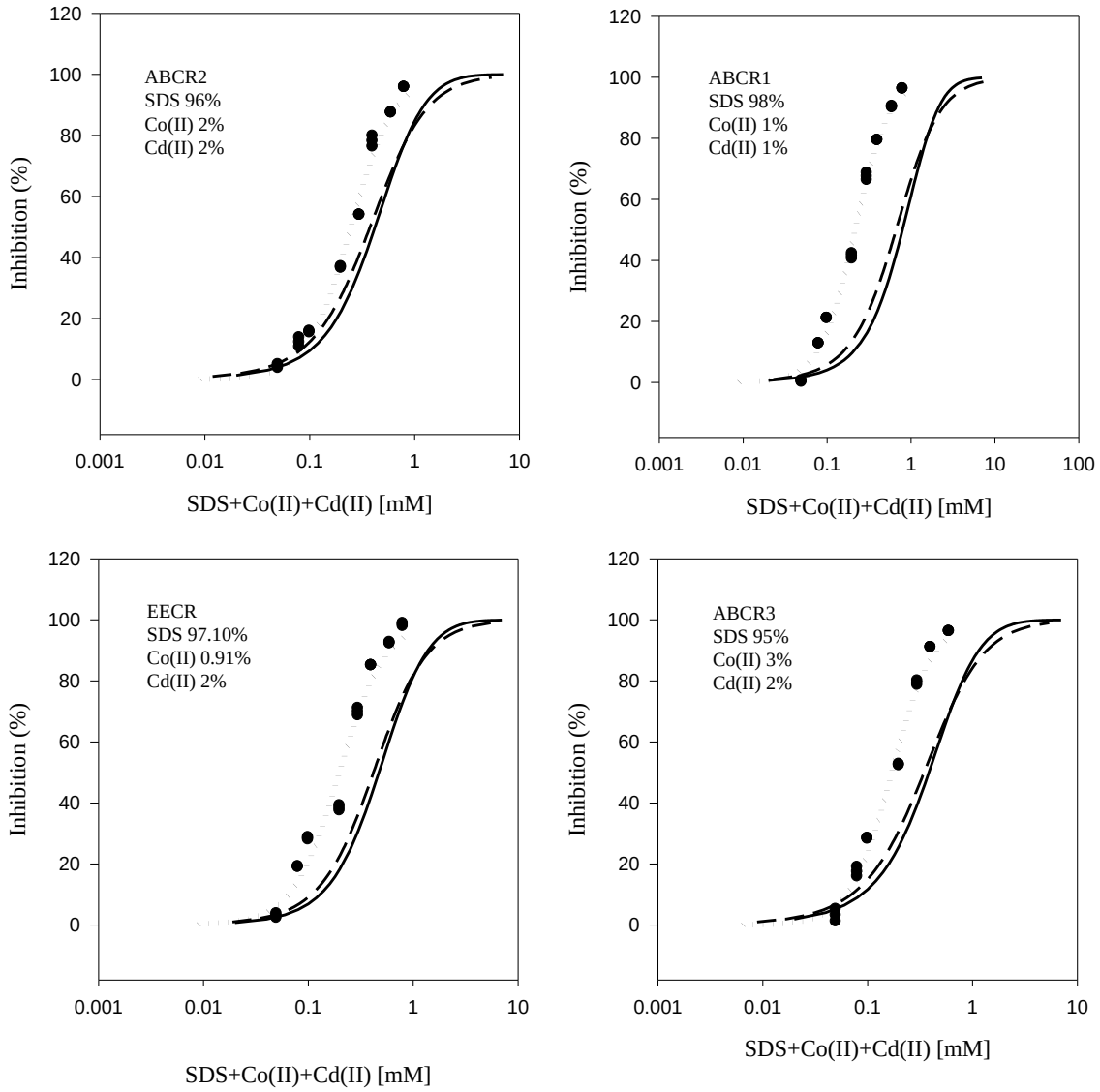


Figure 4.28: Experimental and predicted inhibitory effects of ternary mixtures ofSDS, cobalt and cadmium ions on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.4. Toxicity of quaternary mixtures

4.3.4.1.Toxicity of quaternary mixtures of SDS and metals to*S. marcescens (SerEW01)*

Table 4.14is the experimentally-derived and predicted toxicity thresholds (EC_{50}) of quaternary mixtures of metal ions and SDS on *S. marcescens* (SerEW01). The experimentally-derived EC_{50S} in SDS + Cd(II) +Zn(II) + Pb(II) ranged from 0.100±0.004mM(ABCR2)to 0.142±0.005mM (ABCR1) mixture ratios. Also, in the same mixture type, ABCR1and ABCR3mixture ratios were significantly different from the other mixture ratios.

In SDS+Cd(II)+Co(II)+Pb(II) mixtures, the experimentally-derived $EC_{50}$for ABCR2 mixture ratio was the most toxic (0.074±0.004mM), while ABCR1mixture ratio was the least toxic (0.112±0.003mM).Similarly,ABCR1 andABCR2mixture ratios were statistically different from the other mixture ratios. In SDS+Cd(II)+Ni(II)+Pb(II) mixtures, the experimentally-derived EC_{50S} showed that only ABCR3mixture ratio was statistically different from EECR50 mixture ratio. In addition, in EECR50mixture ratio,the experimental EC_{50}wasstatistically different from both CA and IA-predicted EC_{50S}, while in other mixture ratios,the experimental $EC_{50,}$ CA- and IA-predicted EC_{50S}were statisticallydifferent from oneanother ($P < 0.05$).However, apart from EECR50 mixture ratio of SDS + Cd(II) + Ni(II) + Pb(II) mixture type, in other mixture ratios of the quaternary mixtures, both experimentally-derived $EC_{50S,}$ and EC_{50S} predicted on the basis of CA and IA models were significantly different from one another ($P< 0.05$)

Toxic index, model deviation ratio and effect of metals and SDS quaternary mixtures on *S. marcescens* (SerEW01) are shown in Table 4.15. The toxic index (TI) values ranged from 0.092±0.068 to 0.247 ±0.004, while model deviation ratio (MDR) ranged from 4.041±0.071 to 8.854±0.215 for CA and 6.738±0.270 to 16.394±1.312 for IA.At all the mixture ratiostested, the metals and SDS quaternary mixtures were synergistic in their action on the bacterium. The

experimental dose-response relationships of the quaternary mixtures as well as the predictions made from CA and IA models for *S. marcescens* (SerEW01) are shown in Figures 4.29-4.31. All the quaternary mixtures of SDS and metal ions showed that both CA and IA models greatly predicted lower toxicities at all mixture ratios, compared to the experimentally-data and were equally toxic even at low concentrations.

Table 4.14: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Quaternary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

	EC_{50} (mM)‡ [+]		
Toxicant Quaternary Mixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)			
SDS 92.59%+ Cd(II) 2.12%+ Zn(II) 1.22%+ Pb(II) 4.07% (EECR50)	0.102 ± 0.005a*	0.721 ± 0.033**	1.301 ± 0.040***
SDS 93% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% (ABCR1)	0.142 ± 0.005b*	0.637 ± 0.034**	1.063 ± 0.006***
SDS 91% + Cd(II) 3% + Zn(II) 3% + Pb(II) 3% (ABCR2)	0.100 ± 0.004a *	0.549 ± 0.028**	0.942 ± 0.014***
SDS 90% + Cd(II) 3% + Zn(II) 4% + Pb(II) 3% (ABCR3)	0.122 ± 0.005c *	0.492 ± 0.027**	0,819 ± 0.004***
SDS+Cd(II)+Co(II)+Pb(II)			
SDS 92.04% + Cd(II) 2.22% + Co(II) 1.80%+ Pb(II) 4.05% (EECR50)	0.099 ± 0.004a*	0.754 ± 0.029**	1.407 ± 0.029***
SDS 93% + Cd(II) 2% + Co(II) 3% + Pb(II) 2% (ABCR1)	0.112 ± 0.003b*	0.788 ± 0.028**	1.439 ± 0.025***
SDS 91% + Cd(II) 3% + Co(II) 3% + Pb(II) 3% (ABCR2)	0.074 ± 0.004c *	0.658 ± 0.024**	1.215 ± 0.024***
SDS 90% + Cd(II) 3% + Co(II) 4% + Pb(II) 3% (ABCR3)	0.098 ± 0.005a*	0.613 ± 0.022**	1.130± 0.023***
SDS+Cd(II)+Ni(II)+Pb(II)			
SDS 87.99% +Cd(II) 2.01% + Ni(II) 6.12%+ Pb(II) 3.87% (EECR50)	0.098 ± 0.005ab*	0.026 ± 0.001**	0.026 ± 0.001**
SDS 88% +Cd(II) 3% + Ni(II) 6% + Pb(II) 3% (ABCR1)	0.101 ± 0.002a*	0.569 ± 0.031**	0.892 ± 0.012***
SDS 86% +Cd(II) 4% + Ni(II) 6% + Pb(II) 4% (ABCR2)	0.093 ± 0.003bc*	0.497 ± 0.026**	0.779 ± 0.013***
SDS 87% +Cd(II) 4 + Ni(II) 6% + Pb(II) 3% (ABCR3)	0.088 ± 0.002c*	0.519 ± 0.027**	0.794 ± 0.001***

[+] Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$)

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P< 0.05$).

Table 4.15: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Quaternary Mixtures on *S. marcescens* (SerEW01)

Metal+SDS Quternary Mixtures	Toxic Index (TI)[+]	MDR[+] CA	MDR[+] IA	Effect
SDS +Cd (II)+Zn(II)+Pb(II)				
SDS 92.59% +Cd (II) 2.12%+Zn(II) 1.22%+Pb(II) 4.07% ($EECR_{50}$)	0.142 ± 0.001	7.049 ± 0.054	12.824±1.073	Synergistic
SDS 93% +Cd (II) 2%+Zn(II) 3%+Pb(II) 2% (ABCR1)	0.223 ± 0.004	4.487 ± 0.084	7.490 ± 0.300	Synergistic
SDS 91% +Cd (II) 3%+Zn(II) 3%+Pb(II) 3% (ABCR2)	0.183 ± 0.001	5.471 ± 0.037	9.409 ± 0.556	Synergistic
SDS 90% +Cd (II) 3%+Zn(II) 4%+Pb(II) 3% (ABCR3)	0.247 ± 0.004	4.041 ± 0.071	6.738 ± 0.270	Synergistic
SDS +Cd (II)+Co(II)+Pb(II)				
SDS 92.04% +Cd (II) 2.11%+Co(II) 1.80%+Pb(II) 4.05% ($EECR_{50}$)	0.092 ± 0.068	7.594 ± 0.059	14.189±0.934	Synergistic
SDS 93% +Cd (II) 2%+Co(II) 3%+Pb(II) 2% (ABCR1)	0.142 ± 0.001	7.037 ± 0.065	12.856±0.568	Synergistic
SDS 91% +Cd (II) 3%+Co(II) 3%+Pb(II) 3% (ABCR2)	0.113 ± 0.003	8.854 ± 0.215	16.394±1.312	Synergistic
SDS 90% +Cd (II) 3%+Co(II) 4%+Pb(II) 3% (ABCR3)	0.159 ± 0.002	6.274 ± 0.071	11.594±0.767	Synergistic
SDS +Cd (II)+Ni(II)+Pb(II)				
SDS 87.99% +Cd (II) 2.01%+Ni(II) 6.12%+Pb(II) 3.87% ($EECR_{50}$)	0.164 ± 0.002	6.103 ± 0.063	9.896 ± 0.611	Synergistic
SDS 88% +Cd (II) 3%+Ni(II) 6%+Pb(II) 3% (ABCR1)	0.178 ± 0.006	5.626 ± 0.193	8.848 ± 0.286	Synergistic
SDS 86% +Cd (II) 4%+Ni(II) 6%+Pb(II) 4% (ABCR2)	0.188 ± 0.003	5.325 ± 0.079	8.361 ± 0.448	Synergistic
SDS 87% +Cd (II) 4%+Ni(II) 6%+Pb(II) 3% (ABCR3)	0.170 ± 0.005	5.893 ± 0.175	9.024 ± 0.308	Synergistic

[+]Values are reported as Mean ± 1SD

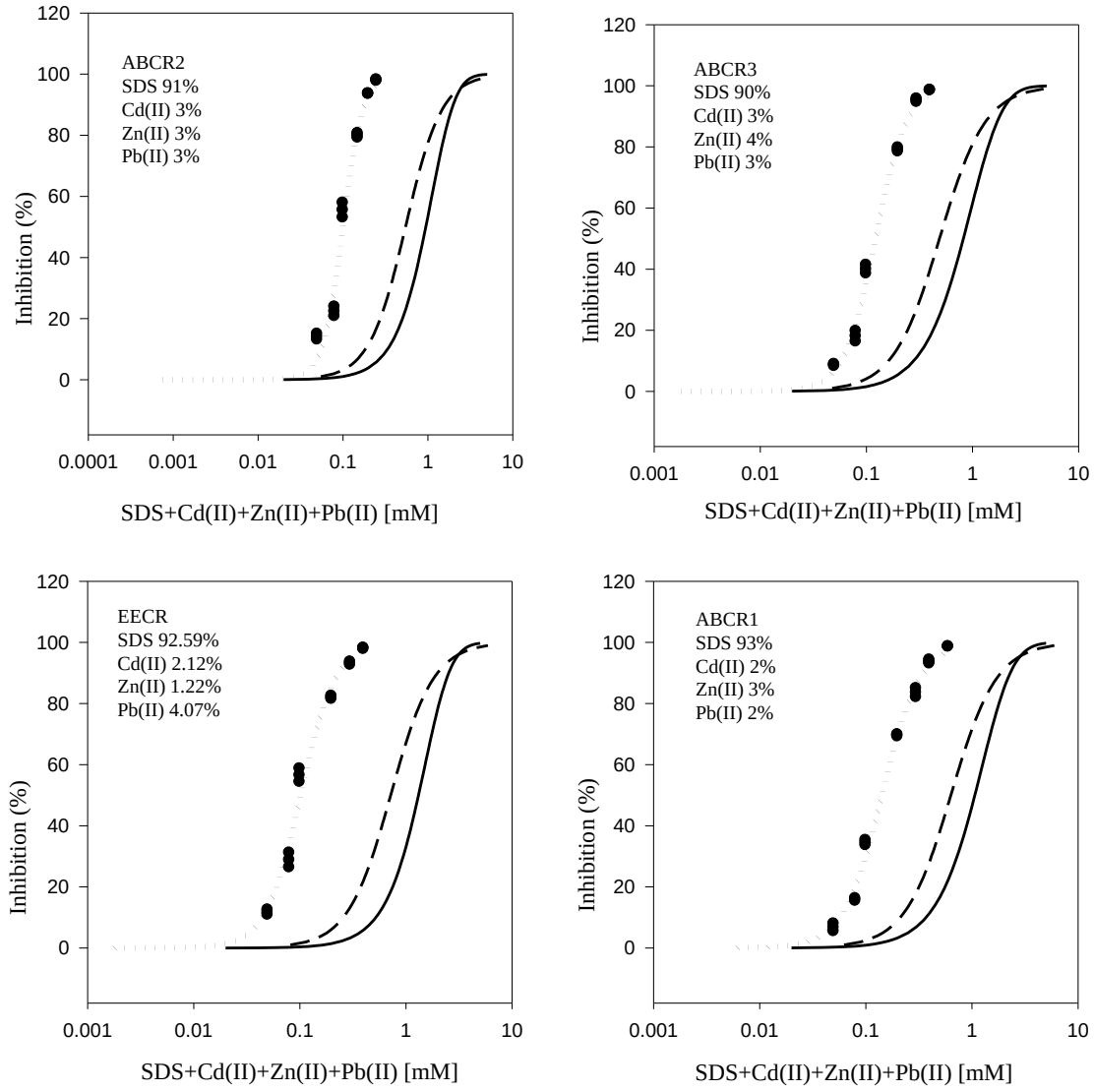


Figure 4.29: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, zinc and lead ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

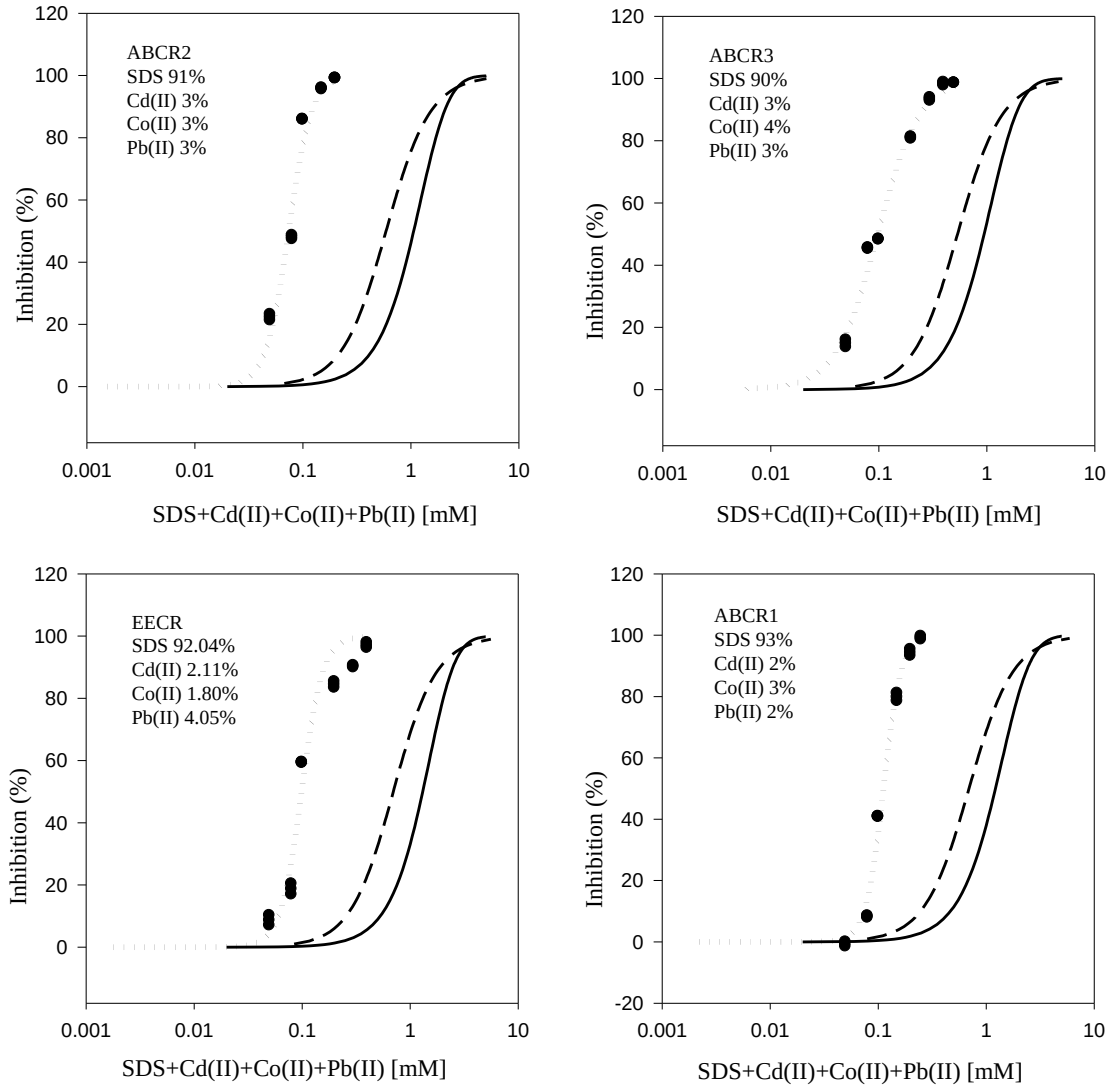


Figure 4.30: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, cobalt and lead ions on *S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

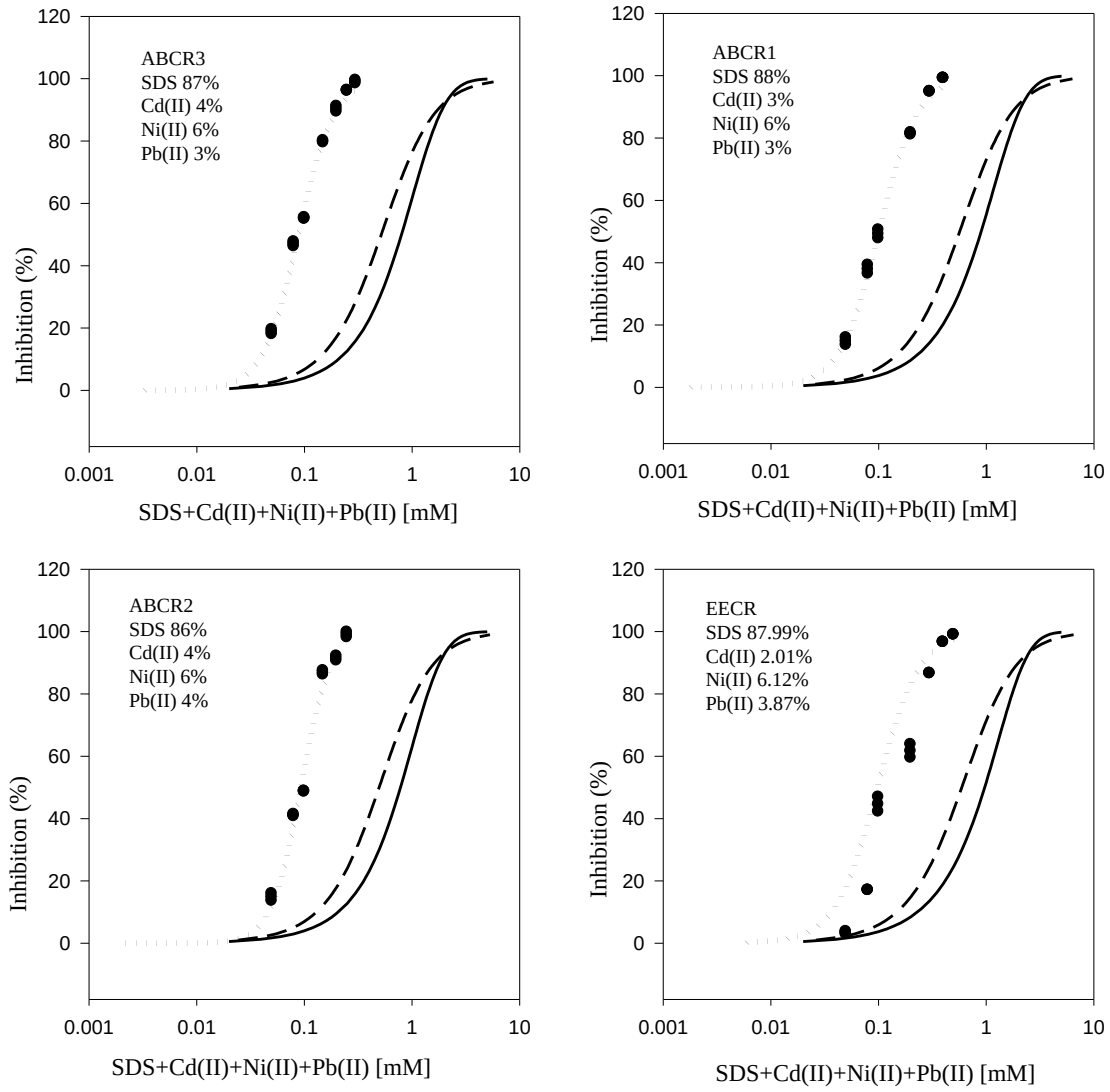


Figure 4.31: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, nickel and lead ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.4.2.Toxicity of quaternary mixtures of SDS and metals to A. seifertii

Table 4.16is the experimentally-derived and predicted toxicity thresholds (EC_{50}) of quaternary mixtures of metals and SDS on *A.seifertii.* The experimentally-derived EC_{50S} in SDS + Cd(II) +Zn(II) + Pb(II)mixture showed that ABCR1mixture ratio had the highest EC_{50}(0.255 ± 0.013 mM) while ABCR3 had the least (0.196±0.014mM). The $EC_{50S}$of ABCR1and ABCR2mixture ratios were significantly different from the others. In SDS+Cd(II)+Co(II)+Pb(II) mixtures, the experimentally-derived EC_{50} values ranged from 0.157±0.006mM (ABCR2) to 0.197±0.011mM (ABCR3)mixture ratios.Onlythe experimentally-derived EC_{50} for ABCR2mixture ratio was statistically different from the other EC_{50S}in the mixture type.Similarly, in SDS+Cd(II)+Ni(II)+Pb(II) quaternary mixture type, only ABCR1mixture ratio was statistically different from EECR50 mixture ratio, within the experimentally-derived EC_{50S},In addition, in all mixture ratios of the quaternary mixtures, the experimentally-derived $EC_{50S}$and those predicted from theCA- and IA-models were statistically different from one another ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS quaternary mixtures on *A.seifertii*are shown in Table 4.17. The toxic index (TI) values ranged from 0.222 ± 0.007 to 0.705 ± 0.023, while model deviation ratio (MDR) ranged from 1.420 ± 0.046to 4.498 ± 0.1398 for CA and 1.792 ± 0.111to 5.559 ± 0.291for IA.At all the mixture ratios tested, the metals and SDS quaternary mixtures were synergistic in their action on the bacterium. The experimental dose-response relationships of the quaternary mixtures as well as the predictions made from CA and IA models for *A.seifertii*are shown in Figures 4.32-4.34. In the quaternary mixtures of SDS + Cd(II) + Zn(II)+Pb(II),the CA model almost correctly predicted the experimentally-derived data at low concentrations. In addition, both CA and IA models slightlypredicted lower

toxicities at lower concentrations,epecially for ABCR1 and ABCR2 mixture ratios, compared to the experimentally-derived data (Figure 4.32). In Figure 4.33, except for EECR50mixture ratio, in theother mixture ratios, CA and IA models slightly overestimated the toxicities at low concentrations, while in all mixture ratios however,the models underestimated the toxicities at higher concentrations. Similarly, except for EECR50 mixture ratio, others predicted slightly lower toxicities than the experimentally-derived data would suggest (Figure 4.34).

Table 4.16: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Quaternary Mixtures of Metals and SDS on *A.seifertii*

EC_{50} (mM)‡ [+]			
Toxicant Quternary Mixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)			
SDS 93.06% + Cd(II) 1.91% + Zn(II) 1.23% + Pb(II) 3.80% (EECR50)	0.201 ± 0.005a*	0.425± 0.021**	0.563 ± 0.102***
SDS 93% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% (ABCR1)	0.255 ± 0.013b*	0.387 ± 0.020**	0.513 ± 0.015***
SDS 92% + Cd(II) 2% + Zn(II) 3% + Pb(II) 3% (ABCR2)	0.233 ± 0.006c *	0.381 ± 0.019**	0.513 ± 0.412***
SDS 91% + Cd(II) 3% + Zn(II) 4% + Pb(II) 2% (ABCR3)	0.196 ± 0.014a*	0.278 ± 0.014**	0.351 ± 0.099***
SDS+Cd(II)+Co(II)+Pb(II)			
SDS 93.39% + Cd(II) 1.92% + Co(II) 0.87% + Pb(II) 3.82% (EECR50)	0.186 ± 0.007a*	0.414 ± 0.025**	0.499 ± 0.079***
SDS 94% + Cd(II) 2% + Co(II) 2% + Pb(II) 2% (ABCR1)	0.186 ± 0.008a*	0.373 ± 0.028**	0.426 ± 0.004***
SDS 93% + Cd(II) 2% + Co(II) 3% + Pb(II) 2% (ABCR2)	0.157 ± 0.006b*	0.342 ± 0.029**	0.388 ± 0.067***
SDS 92% + Cd(II) 2% + Co(II) 4% + Pb(II) 2% (ABCR3)	0.197 ± 0.011a*	0.316 ± 0.029**	0.357 ± 0.040***
SDS+Cd(II)+Ni(II)+Pb(II)			
SDS 90.72% +Cd(II) 1.86% + Ni(II) 3.71% + Pb(II) 3.71% (EECR50)	0.102 ± 0.007a*	0.457 ± 0.022**	0.564 ± 0.119***
SDS 91% +Cd(II) 3% + Ni(II) 4% + Pb(II) 2% (ABCR1)	0.117 ± 0.008b*	0.320 ± 0.016**	0.362 ± 0.017***
SDS 89% +Cd(II) 3% + Ni(II) 5% + Pb(II) 3% (ABCR2)	0.113 ± 0.002ab*	0.315 ± 0.015**	0.359 ± 0.127***
SDS 90% +Cd(II) 3% + Ni(II) 4% + Pb(II) 3% (ABCR3)	0.112 ± 0.006ab*	0.316 ± 0.016**	0.362 ± 0.014***

[+]Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$)

‡ Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P < 0.05$).

Table 4.17: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Quaternary Mixtures on *A.seifertii*

Metal+SDS Quternary Mixtures	Toxic Index (TI)[+]	MDR[+]		Effect
		CA	IA	
SDS +Cd (II)+Zn(II)+Pb(II)				
SDS 93.06% +Cd (II) 1.91%+Zn(II) 1.23%+Pb(II) 3.80% ($EECR_{50}$)	0.474 ± 0.011	2.112 ± 0.051	2.797 ± 0.054	Synergistic
SDS 93% +Cd (II) 2%+Zn(II) 3%+Pb(II) 2% (ABCR1)	0.658 ± 0.013	1.519 ± 0.029	2.016 ± 0.090	Synergistic
SDS 92% +Cd (II) 2%+Zn(II) 3%+Pb(II) 3% (ABCR2)	0.612 ± 0.017	1.634 ± 0.045	2.201 ± 0.054	Synergistic
SDS 91% +Cd (II) 3%+Zn(II) 4%+Pb(II) 2% (ABCR3)	0.705 ± 0.023	1.420 ± 0.046	1.792 ± 0.111	Synergistic
SDS +Cd (II)+Co(II)+Pb(II)				
SDS 93.39% +Cd (II) 1.92%+Co(II) 0.87%+Pb(II) 3.82% ($EECR_{50}$)	0.450 ± 0.010	1.761 ± 1.047	2.683 ± 0.118	Synergistic
SDS 94% +Cd (II) 2%+Co(II) 2%+Pb(II) 2% (ABCR1)	0.501 ± 0.014	1.594 ± 0.937	2.288 ± 0.113	Synergistic
SDS 93% +Cd (II) 2%+Co(II) 3%+Pb(II) 2% (ABCR2)	0.460 ± 0.022	2.183 ± 0.107	2.486 ± 0.083	Synergistic
SDS 92% +Cd (II) 2%+Co(II) 4%+Pb(II) 2% (ABCR3)	0.626 ± 0.023	1.600 ± 0.060	1.818 ± 0.086	Synergistic
SDS +Cd (II)+Ni(II)+Pb(II)				
SDS 90.72% +Cd (II) 1.86%+Ni(II) 3.71%+Pb(II) 3.71% ($EECR_{50}$)	0.222 ± 0.007	4.498 ± 0.139	5.559 ± 0.291	Synergistic
SDS 91% +Cd (II) 3%+Ni(II) 4%+Pb(II) 2% (ABCR1)	0.364 ± 0.012	2.747 ± 0.089	3.104 ± 0.143	Synergistic
SDS 89% +Cd (II) 3%+Ni(II) 5%+Pb(II) 3% (ABCR2)	0.359 ± 0.012	2.785 ± 0.094	3.178 ± 0.074	Synergistic
SDS 90% +Cd (II) 3%+Ni(II) 4%+Pb(II) 3% (ABCR3)	0.354 ± 0.008	2.822 ± 0.067	3.178 ± 0.074	Synergistic

[+]Values are reported as Mean ± 1SD

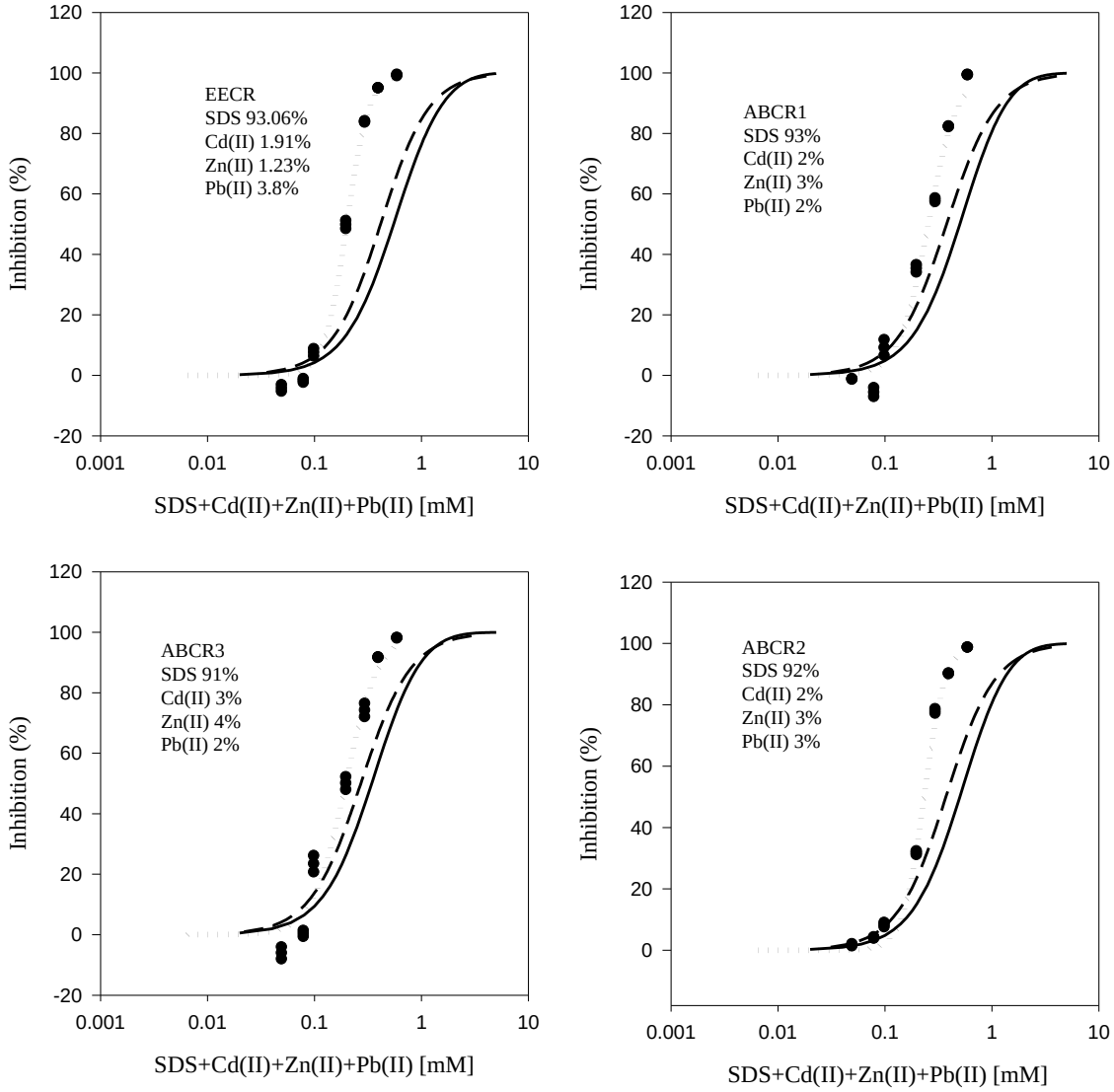


Figure 4.32: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, zinc and lead ions on *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

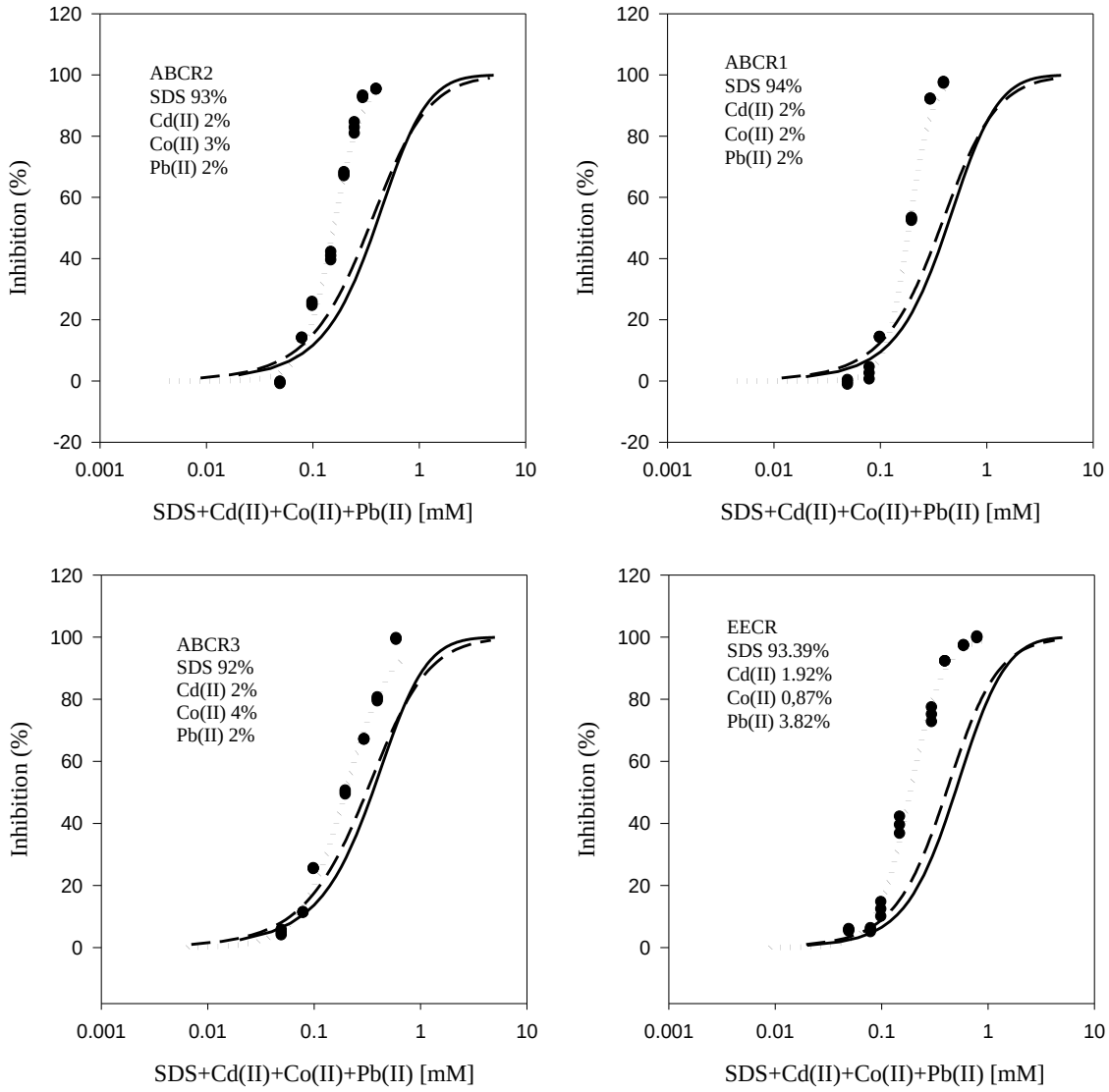


Figure 4.33: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, cobalt and lead ionson *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2) or hormetic model (Eqn. 3). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

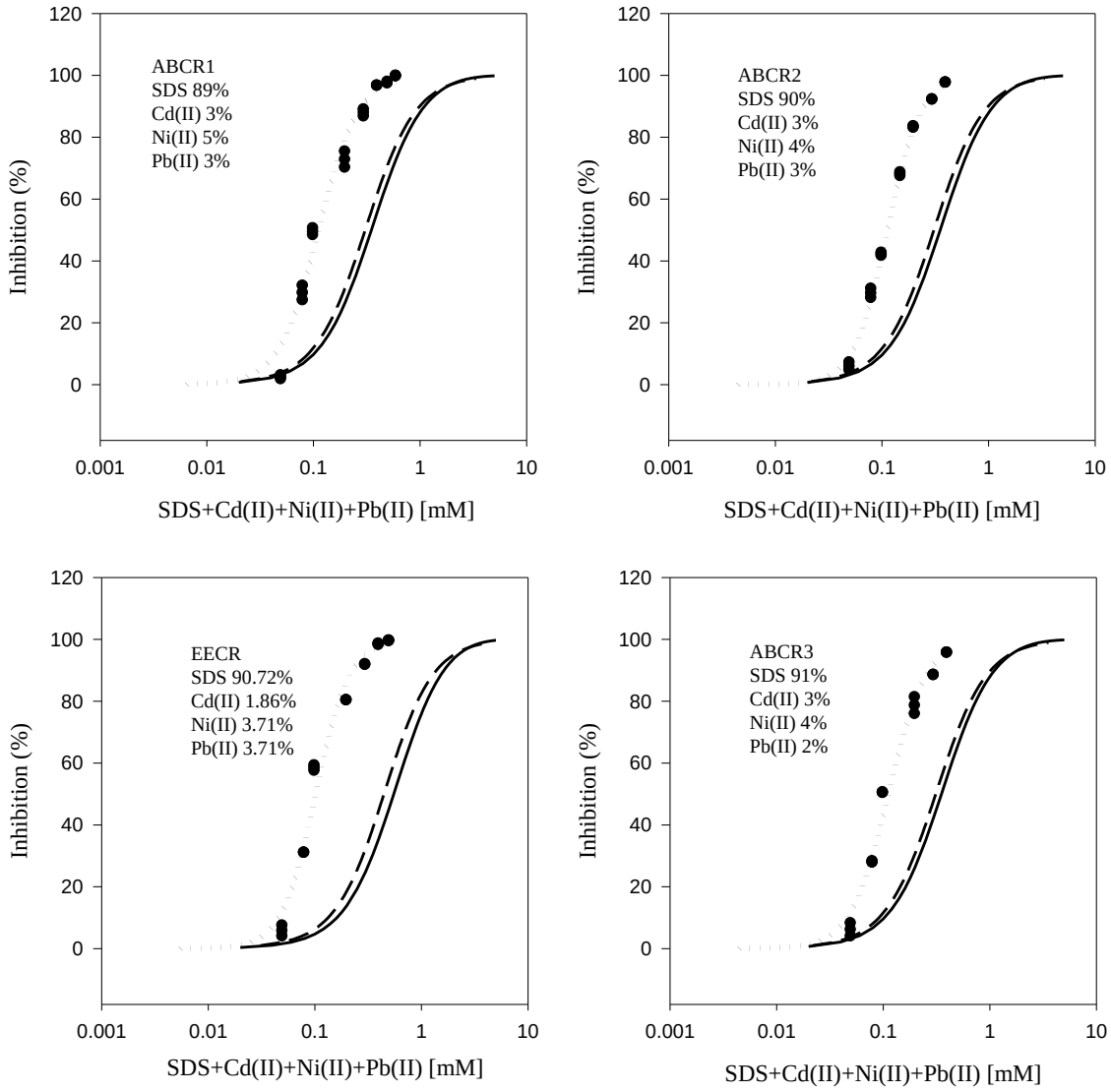


Figure 4.34: Experimental and predicted inhibitory effects of quaternary mixtures ofSDS, cadmium, nickel and lead ions on *A.rseifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.5. Toxicity of quinary mixtures

4.3.5.1.Toxicity of quinary mixtures of SDS and metals to*S. marcescens (SerEW01)*

Table 4.18is the experimental and predicted toxicity thresholds (EC_{50}) of quinary mixtures of metals and SDS on *S. marcescens* (SerEW01). The experimentally-derived EC_{50S} in SDS + Cd(II) + Zn(II) + Pb(II) + Co(II)quinary mixture ranged from 0.107±0.004mM (ABCR2 and ABCR3)to 0.129±0.007mM (ABCR1) mixture ratios. The experimentally-derived $EC_{50S}$for ABCR1mixture ratios was significantly higher thanthe others. Similarly, in quinary mixturesof SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II), the experimentally-derived EC_{50S}showed no statistical difference from oneanother. In addition, ABCR1 and ABCR2 mixture ratios were the most toxic (0.120 ± 0,007 mM), while EECR50 mixture ratio was the least (0.125 ± 0.008 mM).

In SDS+Cd(II)+Zn(II)+Ni(II)+Co(II) quinary mixtures, within the experimentally-derived $EC_{50S,}$ ABCR2was the most toxic mixture ratio (0.113 ± 0.006 mM), while ABCR1 mixture ratio was the least (0.133 ± 0.011 mM). In addition,in all mixture ratios of the quinary mixture type, the experimentally-derived$EC_{50S,}$ CA- and IA-predicted EC_{50S}, were statistically different from eone another ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS quinary mixtures on *Serratia marcescens* (SerEW01) are shown in Table 4.19. The toxic index (TI) values ranged from 0.178±0.003 to 0.386±0.002, while model deviation ratio (MDR) ranged from 3.105±0.023 to 5.620±0.098 for CA and 4.739±0.299 to 11.331±0.717 for IA.At all the mixture ratios tested, the metals and SDS quinary mixtures were synergistic in their actions on the bacterium.

The experimental dose-response relationships of the quinary mixtures as well as the predictions made from CA and IA models for *S. marcescens* (SerEW01) are shown in Figures 4.35-4.37.

All the quinary mixtures of SDS and metal ions showed that both CA and IA models greatly predicted lower toxicities at all mixture ratios, compared to the experimentally-derived data and were toxic even at low concentrations.

Table 4.18: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Quinary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

	EC_{50} (mM)‡ [+]		
Toxicant Quinary Mixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)			
SDS 90.90% + Cd(II) 2.10% +Zn(II) 1.20% + Pb(II) 4.0% + Co(II) 1.80% (EECR 50)	0.113 ± 0.003a*	0.635 ± 0.028**	1.279 ±0.047***
SDS 91% + Cd(II) 2% + Zn(II) 2% + Pb(II) 2% + Co(II) 3% (ABCR1)	0.129 ± 0.007b*	0.591 ± 0.027**	1.153 ± 0.034***
SDS 89% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% + Co(II) 4% (ABCR2)	0.107 ± 0.004a*	0.496 ± 0.024**	0.937 ± 0.020c***
SDS 88% + Cd(II) 3% + Zn(II) 4% + Pb(II) 1% + Co(II) 4% (ABCR3)	0.107 ± 0.004a*	0.433 ± 0.022**	0.766 ± 0.001***
SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II			
SDS 86.99% + Cd(II) 1.99% + Ni(II) 6.06% + Pb(II) 3.83% + Zn(II) 1.14% (EECR 50)	0.125 ± 0.008a*	0.525 ± 0.031**	0.904 ± 0.030***
SDS 87% + Cd(II) 2% + Ni(II) 6% + Pb(II) 2% + Zn(II) 3% (ABCR1)	0.120 ± 0.008a*	0.467 ± 0.029**	0.743 ± 0.019***
SDS 86% + Cd(II) 2% + Ni(II) 4% + Pb(II) 2% + Zn(II) 6% (ABCR3)	0.120 ± 0.007a*	0.366 ± 0.022**	0.559 ± 0.002***
SDS+Cd(II)+Zn(II)+Ni(II)+Co(II)			
SDS 88.87% + Cd(II) 2.03% + Zn(II) 1.17% + Ni(II) 6.19% + Co(II) 1.74% (EECR 50)	0.124 ± 0.010ab*	0.555 ± 0.032**	0.914 ± 0.023***
SDS 89% + Cd(II) 2% + Zn(II) 4% + Ni(II) 2% + Co(II) 3% (ABCR1)	0.133 ± 0.011b*	0.468 ± 0.026**	0.778 ± 0.015***
SDS 87% + Cd(II) 1% + Zn(II) 5% + Ni(II) 4% + Co(II) 2% (ABCR2)	0.113 ± 0.006a*	0.412 ± 0.025**	0.6333 ± 0.007***
SDS 86 + Cd(II) 2% + Zn(II) 6% + Ni(II) + 2% + Co(II) 3% (ABCR3)	0.118 ± 0.006ab*	0.366 ± 0.021**	0.579 ± 0.003***

[+]Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$)

‡Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P < 0.05$).

Table 4.19: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Quinary Mixtures on *S. marcescens* (SerEW01)

Metal+SDS Quinary Mixtures	Toxic Index (TI)[+]	MDR[+]		
		CA	IA	Effect
SDS +Cd (II)+Zn(II)+Pb(II)+Co(II)				
SDS 90.90% +Cd (II) 2.10%+Zn(II) 1.20%+Pb(II) 4.0% +Co(II) 1.80% (EECR50)	0.178 ± 0.003	5.620 ± 0.098	11.331±0.717	Synergistic
SDS 91% +Cd (II) 2%+Zn(II) 2%+Pb(II) 2% +Co(II) 3% (ABCR1)	0.219 ± 0.003	4.572 ± 0.055	8.945 ± 0.780	Synergistic
SDS 89% +Cd (II) 2%+Zn(II) 3%+Pb(II) 2% +Co(II) 4% ABCR2)	0.216 ± 0.002	4.636 ± 0.051	8.770 ± 0.510	Synergistic
SDS 88% +Cd (II) 3%+Zn(II) 4%+Pb(II) 1% +Co(II) 4% ABCR3)	0.205 ± 0.002	4.879 ± 0.058	8.651 ± 0.433	Synergistic
SDS +Cd (II)+Ni(II)+Pb(II)+Zn(II)				
SDS 86.99% +Cd (II) 1.99%+Ni(II) 6.06%+Pb(II) 3.83% +Zn(II) 1.14% (EECR50)	0.314 ± 0.009	4.188 ± 0.039	7.248 ± 0.725	Synergistic
SDS 87% +Cd (II) 2%+Ni(II) 6%+Pb(II) 2% +Zn(II) 3% ABCR1)	0.331 ± 0.003	3.892 ± 0.019	6.220 ± 0.569	Synergistic
SDS 86% +Cd (II) 2%+Ni(II) 4%+Pb(II) 2% +Zn(II) 6% ABCR3)	0.386 ± 0.002	3.214 ± 0.007	4.739 ± 0.299	Synergistic
SDS +Cd (II)+Zn(II)+Ni(II)+Co(II)				
SDS 88.87% +Cd (II) 2.03%Zn(II) 1.17%+Ni(II) 6.19% +Co(II) 1.74% (EECR50)	0.295 ± 0.006	4.489 ± 0.089	7.432 ± 0.759	Synergistic
SDS 89% +Cd (II) 2%Zni(II) 4%+Ni(II) 2% +Co(II) 3% ABCR1)	0.353 ± 0.009	3.522 ± 0.097	5.883 ± 0.596	Synergistic
SDS 87% +Cd (II) 1%Zni(II) 5%+Ni(II) 4% +Co(II) 2% ABCR2)	0.332 ± 0.001	3.643 ± 0.031	5.618 ± 0.360	Synergistic
SDS 86% +Cd (II) 2%Zni(II) 6%+Ni(II) 2% +Co(II) 3% ABCR3)	0.377 ± 0.001	3.105 ± 0.023	4.919 ± 0.271	Synergistic

[+]Values are reported as Mean ± 1SD

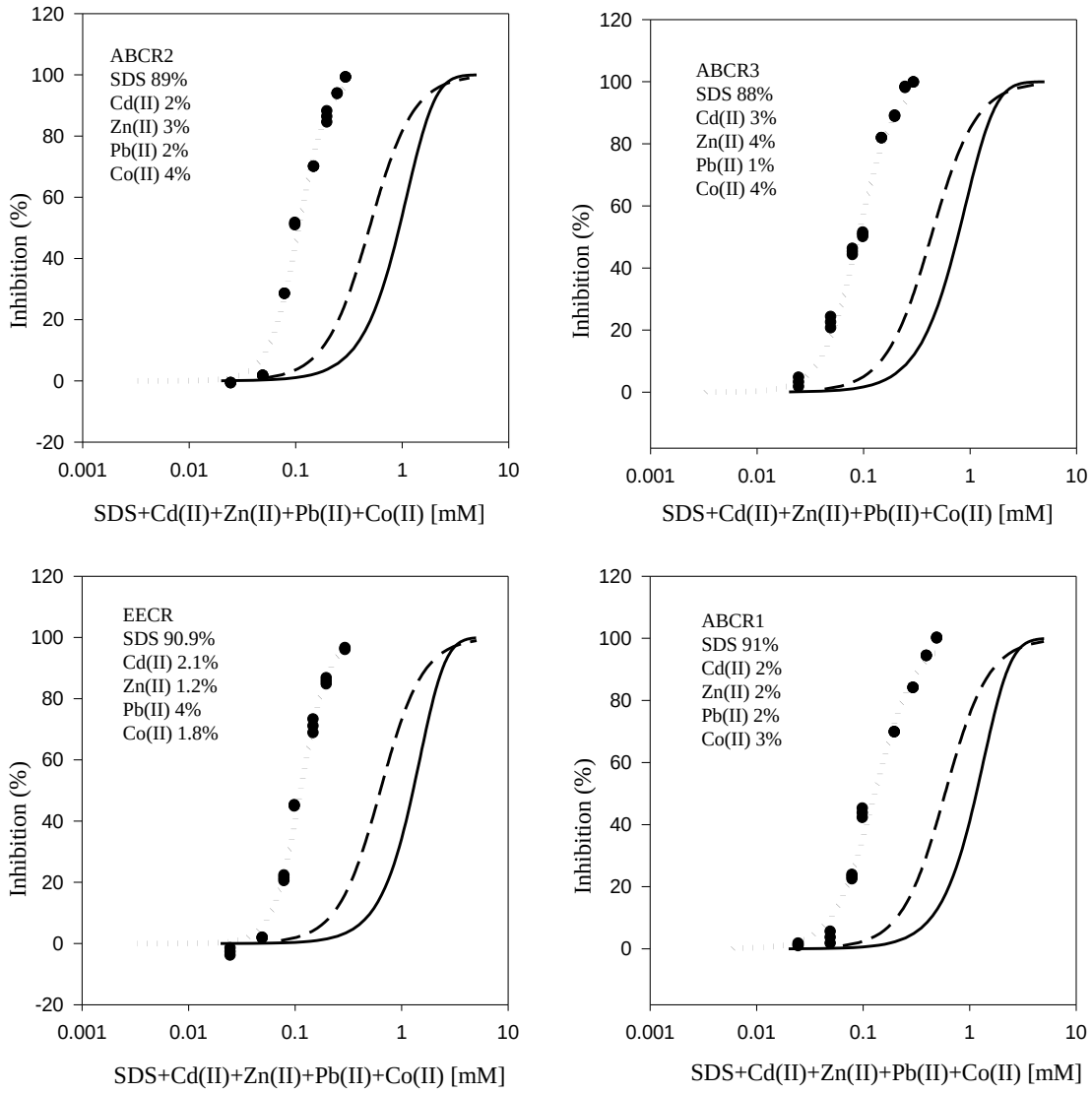


Figure 4.35: Experimental and predicted inhibitory effects of quinary mixtures ofSDS, cadmium, zinc, lead and cobalt ions on *S. marcescens* (SerEW01)dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

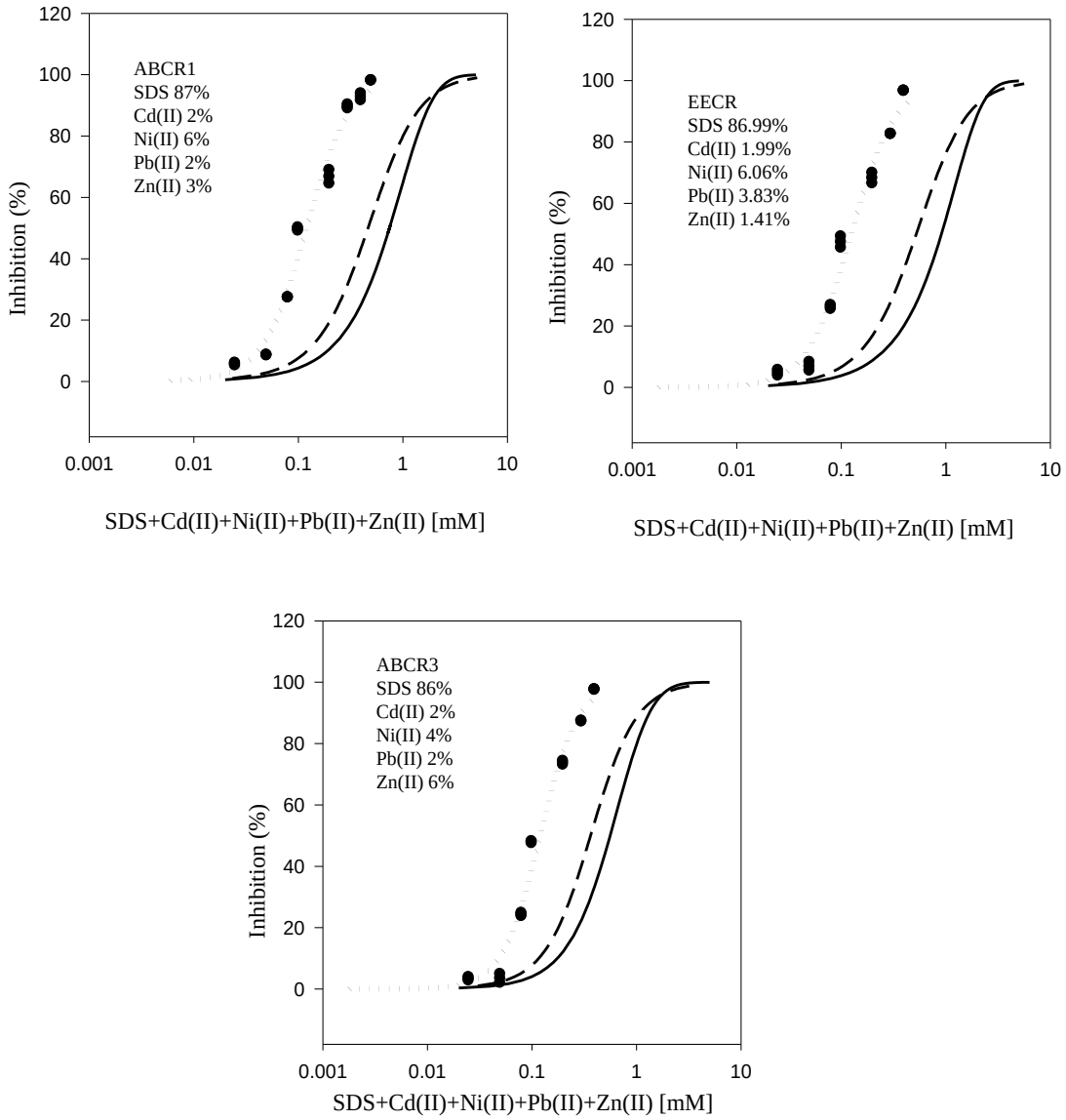


Figure 4.36: Experimental and predicted inhibitory effects of quinary mixtures ofSDS, cadmium, nickel, lead and zinc ions on *S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

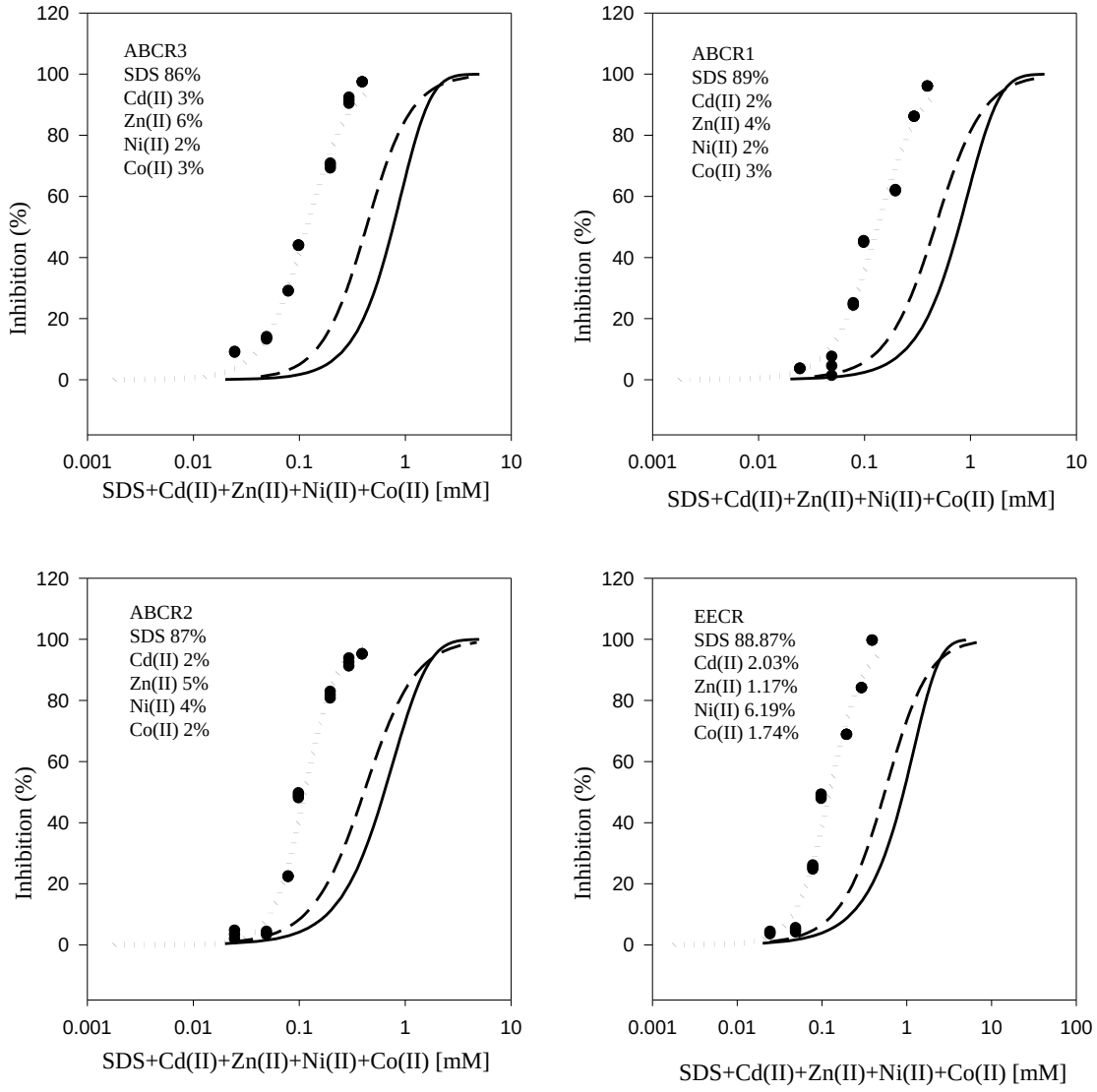


Figure 4.37: Experimental and predicted inhibitory effects of quinary mixtures ofSDS, cadmium, zinc, nickel and cobalt ions on *S. marcescens* (SerEW01)dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.5.2.Toxicity of quinary mixtures of SDS and metals to*A.seifertii*

Table 4.20is the experimentally derived and predicted toxicity thresholds (EC_{50}) of the quinary mixtures of metals and SDS on *A.seifertii*.*T*he experimentally-derived EC_{50S} in of SDS +Cd(II) + Zn(II) + Pb(II) + Co(II)mixture ranged from 0.123±0.005mM (ABCR1)to 0.142±0.004mM (ABCR3) mixture ratios. The $EC_{50S}$of EECR50and ABCR1mixture ratios were significantly different from those of ABCR2 and ABCR3mixture ratios. In SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II) mixtures, within the experimentally-derived $EC_{50S,}$EECR50 mixture ratio was the most toxic (0.113±0.003mM), while ABCR3 mixture ratio recorded the least toxicity (0.283±0.016mM).

In SDS+Cd(II)+Zn(II)+Ni(II)+Co(II) mixtures, the experimentally-derived EC_{50S}showed that EECR50and ABCR1mixture ratios were statistically different from the other mixture ratios. However, in all mixture ratios of the quinary mixture types, the experimentally-derivedEC_{50S}, CA- and IA-predicted EC_{50S}were statistically different from oneanother ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS quinary mixtures on *A.seifertii*are shown in Table 4.21. The toxic index (TI) values ranged from 0.192 ± 0.010 to 0.527 ± 0.009, while model deviation ratio (MDR) ranged from 1.969 ± 0.0290 to 3.834 ± 0.076 for CA and2.667 ± 0.115 to 4.885 ± 0.367 for IA.At all the mixture ratios tested, the metals and SDS quinary mixtures were synergistic in their action on the bacterium.

The experimental dose-response relationships of the quinary mixtures as well as the predictions made from CA and IA models for *A.seifertii* are shown in Figures 4.38-4.40. The quinary mixtures of SDS + Cd(II) + Zn(II) + Pb(II) + Co(II) showed that both CAand IA-models slightly underestimated the toxicities relative to the experimentally-derived data, especially for ABCR2 and ABCR3 mixture ratios. In addition, ABCR2mixture ratio was stimulatory to *A.seifertii's*dehydrogenase activity at low concentrations of up to 0.05mM and inhibitory at

higher concentrations (hormesis). Other mixture ratioswere however inhibitory, even at low concentrations (Figure 4.38). In Figures 4.39 and 4.40, both CA and IA-models slightly predicted lower toxicities and were also inhibitory, even at lower concentrations.

Table 4.20: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Quinary Mixtures of Metals and SDS on *A.seifertii*

EC_{50} (mM)‡ [+]			
Toxicant Quinary Mixtures	**Experimental†**	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)			
SDS 92.25% + Cd(II) 1.89% +Zn(II) 1.22% + Pb(II) 3.77% + Co(II) 0.86% (EECR50)	0.128 ± 0.016a*	0.394 ± 0.024**	0.500 ± 0.069***
SDS 93% + Cd(II) 1% + Zn(II) 2% + Pb(II) 1% + Co(II) 3% (ABCR1)	0.123 ± 0.005a*	0.442 ± 0.044**	0.571 ± 0.004***
SDS 91% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% + Co(II) 2% (ABCR2)	0.140 ± 0.006b*	0.326 ± 0.024**	0.406 ± 0.056***
SDS 90% + Cd(II) 2% + Zn(II) 4% + Pb(II) 2% + Co(II) 2% (ABCR3)	0.142 ± 0.004b*	0.312 ± 0.023**	0.395 ± 0.103***
SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II			
SDS 89.64% + Cd(II) 1.84% + Ni(II) 3.67% + Pb(II) 3.67% + Zn(II) 1.19% (EECR50)	0.113 ± 0.003a*	0.431 ± 0.022**	0.561 ± 0.101***
SDS 90% + Cd(II) 2% + Ni(II) 3% + Pb(II) 1% + Zn(II) 4% (ABCR1)	0.149 ± 0.012b*	0.369 ± 0.020**	0.481 ± 0.008***
SDS 88% + Cd(II) 3% + Ni(II) 3% + Pb(II) 2% + Zn(II) 4% (ABCR2)	0.129 ± 0.006a*	0.276 ± 0.014**	0.344 ± 0.132***
SDS 89% + Cd(II) 1% + Ni(II) 4% + Pb(II) 3% + Zn(II) 3% (ABCR3)	0.283 ± 0.016c*	0.557 ± 0.029**	0.819 ± 0.187***
SDS+Cd(II)+Zn(II)+Ni(II)+Co(II)			
SDS 92.25% + Cd(II) 1.89% + Zn(II) 1.22% + Ni(II) 3.78% + Co(II) 0.86% (EECR50)	0.114 ± 0.007a*	0.412 ± 0.026**	0.482 ± 0.191***
SDS 93% + Cd(II) 1% + Zn(II) 3% + Ni(II) 2% + Co(II) 1% (ABCR1)	0.225 ± 0.011b*	0.529 ± 0.038**	0.694 ± 0.019***
SDS 92% + Cd(II) 1% + Zn(II) 2% + Ni(II) 2% + Co(II) 3% (ABCR2)	0.157 ± 0.013c*	0.445 ± 0.044**	0.556 ± 0.045***
SDS 91% + Cd(II) 2% + Zn(II) 2% + Ni(II) 3% + Co(II) 2% (ABCR3)	0.150 ± 0.008c*	0.346 ± 0.026**	0.405 ± 0.153***

[+]Values are reported as Mean + 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$)

‡Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P < 0.05$).

Table 4.21: Toxic Index, Model Deviation Ratio and Effect of Metals and SDS Quinary Mixtures on *A.seifertii*

Metal+SDS Quinary Mixtures	Toxic Index (TI)[+]	MDR[+] CA	IA	Effect
SDS +Cd (II)+Zn(II)+Pb(II)+Co(II)				
SDS 92.25% +Cd (II) 1.89%+Zn(II) 1.22%+Pb(II) 3.77% +Co(II) 0.86% (EECR 50)	0.261 ± 0.005	3.834 ± 0.076	4.885 ± 0.367	Synergistic
SDS 93% +Cd (II) 1%+Zn(II) 2%+Pb(II) 1% +Co(II) 3% (ABCR1)	0.192 ± 0.010	3.587 ± 0.207	4.648 ± 0.213	Synergistic
SDS 91% +Cd (II) 2%+Zn(II) 3%+Pb(II) 2% +Co(II) 2% (ABCR2)	0.430 ± 0.015	2.329 ± 0.084	2.914 ± 0.176	Synergistic
SDS 90% +Cd (II) 2%+Zn(II) 4%+Pb(II) 2% +Co(II) 2% (ABCR3)	0.457 ± 0.019	2.193 ± 0.092	2.782 ± 0.156	Synergistic
SDS +Cd (II)+Ni(II)+Pb(II)+Zn(II)				
SDS 89.64% +Cd (II) 1.84%+Ni(II) 3.67%+Pb(II) 3.67% +Zn(II) 1.19% (EECR50)	0.333 ± 0.008	3.681 ± 0.087	4.802 ± 0.261	Synergistic
SDS 90% +Cd (II) 2%+Ni(II) 3%+Pb(II) 1% +Zn(II) 4% (ABCR1)	0.454 ± 0.015	2.483 ± 0.090	3,241 ± 0.285	Synergistic
SDS 88% +Cd (II) 3%+Ni(II) 3%+Pb(II) 2% +Zn(II) 4% (ABCR2)	0.527 ± 0.009	2.136 ± 0.041	2.667 ± 0.115	Synergistic
SDS 89% +Cd (II) 1%+Ni(II) 4%+Pb(II) 3% +Zn(II) 3% (ABCR3)	0.517 ± 0.008	1.969 ± 0.029	2.904 ± 0.234	Synergistic
SDS +Cd (II)+Zn(II)+Ni(II)+Co(II)				
SDS 92.25% +Cd (II) 1.89%Zn(II) 1.22%+Ni(II) 3.78% +Co(II) 0.86% (EECR50)	0.338 ± 0.005	3.621 ± 0.070	4.253 ± 0.341	Synergistic
SDS 93% +Cd (II) 1%Zn(II) 3%+Ni(II) 2% +Co(II) 1% (ABCR1)	0.450 ± 0.010	2.354 ± 0.065	3.098 ± 0.287	Synergistic
SDS 92% +Cd (II) 1%Zn(II) 2%+Ni(II) 2% +Co(II) 3% (ABCR2)	0.401 ± 0.005	2.829 ± 0.041	3.557 ± 0.358	Synergistic
SDS 91% +Cd (II) 2%Zn(II) 2%+Ni(II) 3% +Co(II) 2% (ABCR3)	0.485 ± 0.009	2.301 ± 0.057	2.707 ± 0.210	Synergistic

[+]Values are reported as Meas± 1SD

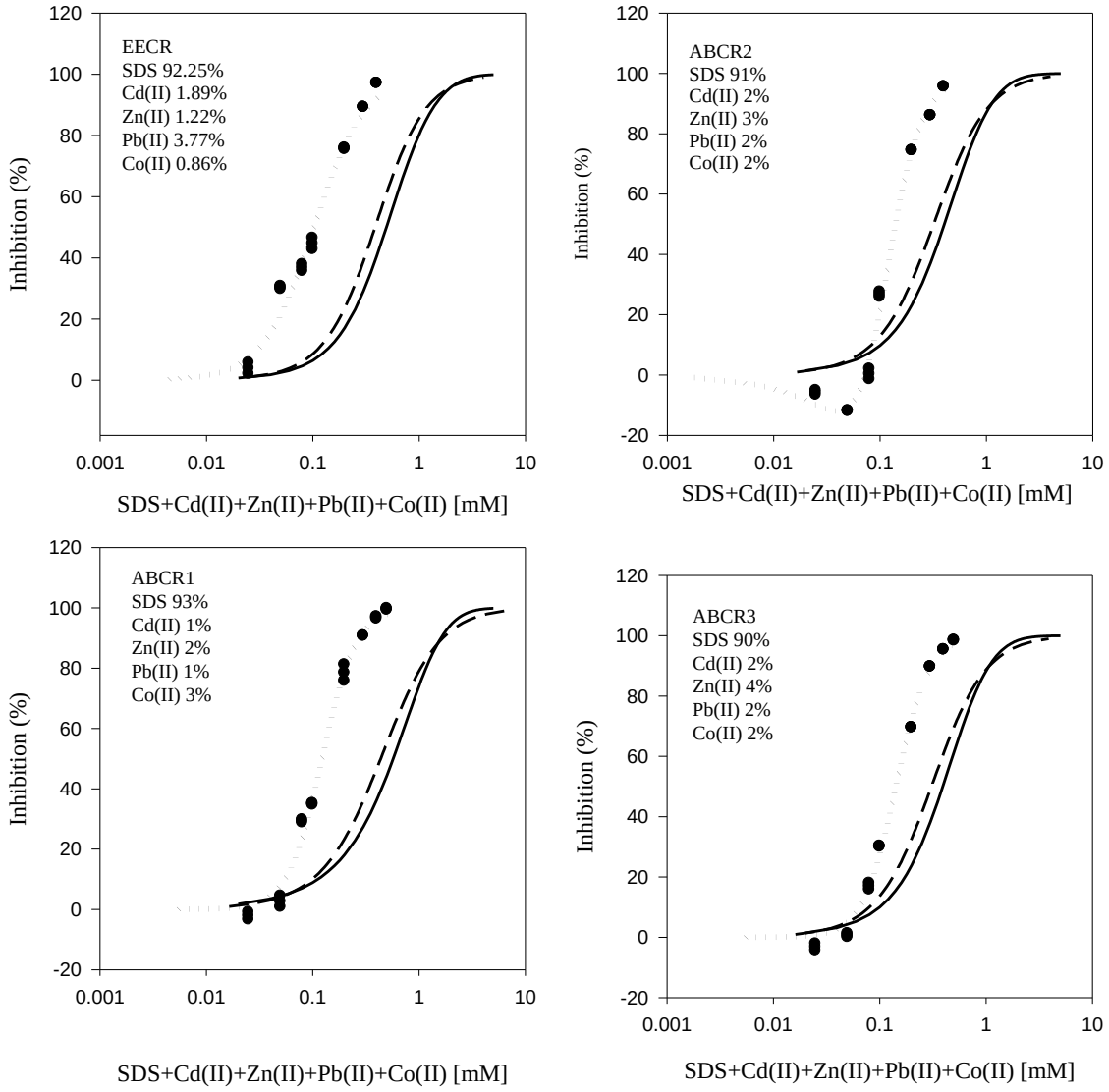


Figure 4.38: Experimental and predicted inhibitory effects of quinary mixtures ofSDS, cadmium, zinc, lead and cobalt ions on *A.seifertii* dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2) or hormetic model (Eq. 3). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

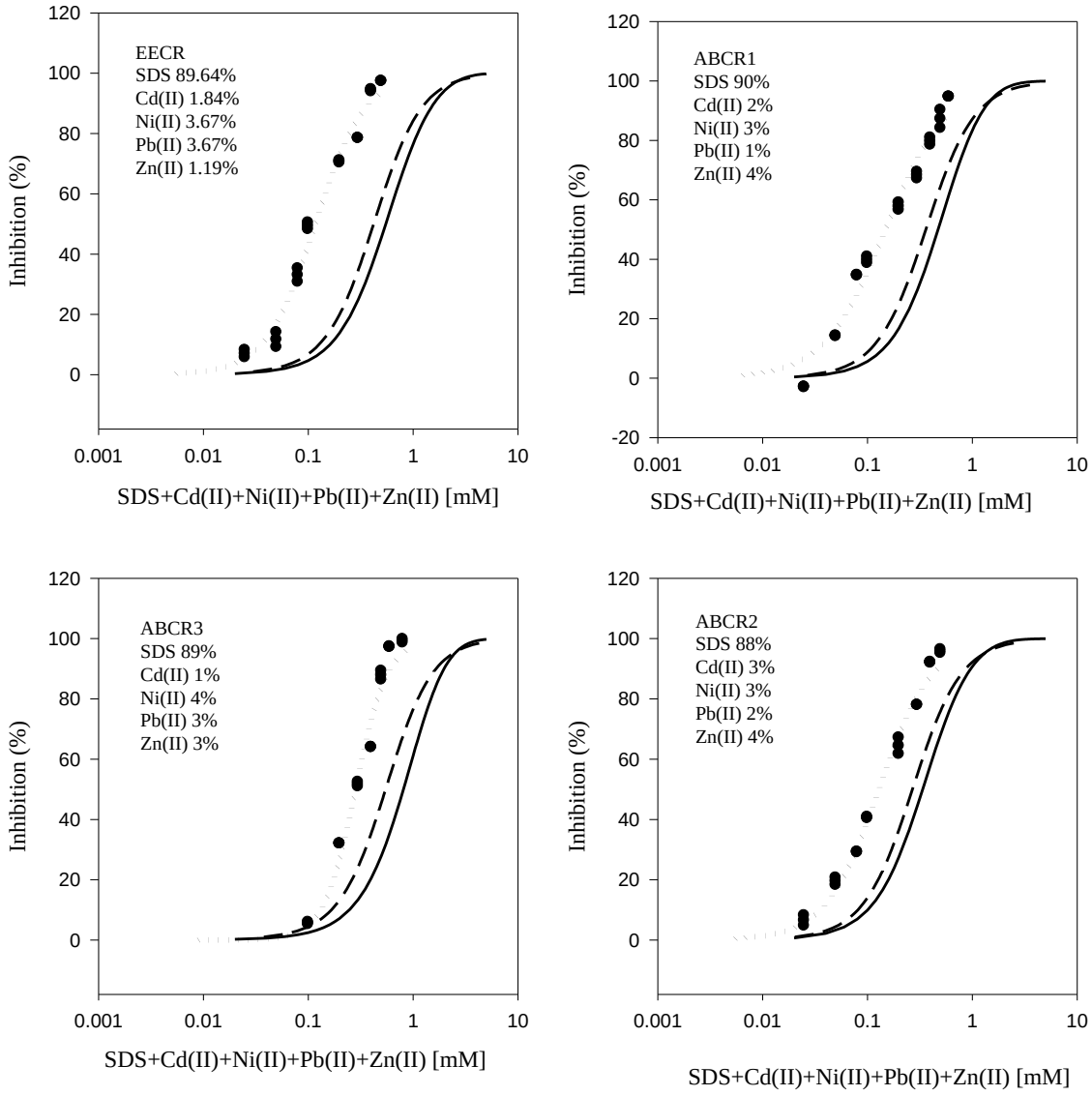


Figure 4.39: Experimental and predicted inhibitory effects of quinary mixtures ofSDS, cadmium, nickel, lead and zinc ions on*A.seifertii* dehydrogenase activity. The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

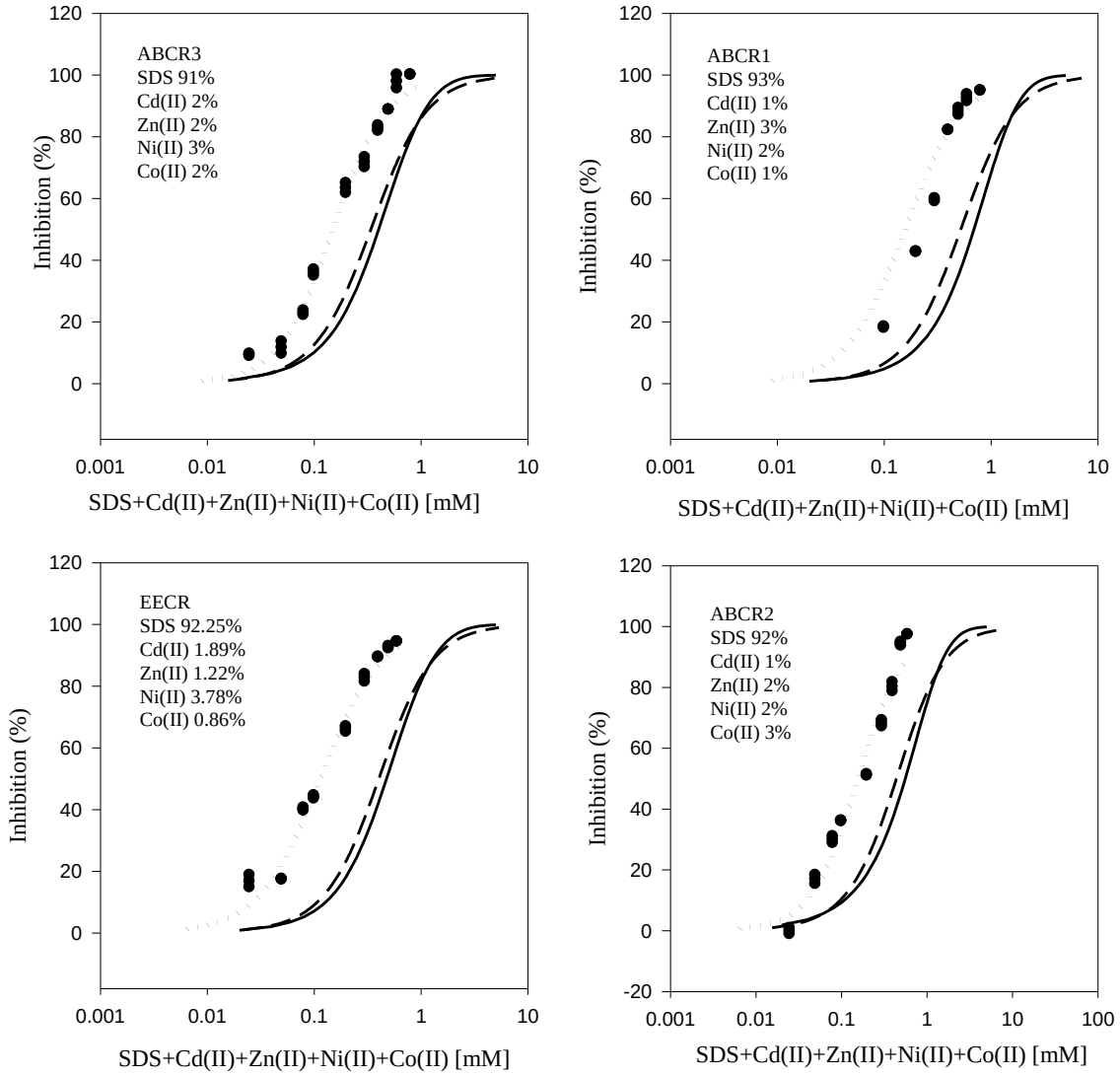


Figure 4.40: Experimental and predicted inhibitory effectsof quinary mixtures ofSDS, cadmium, zinc, nickel and cobalt ions on *A.seifertii* dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.6. Toxicity of senary mixtures

4.3.6.1.Toxicity of senary mixtures of SDS and metal ions to *S. marcescens(SerEW01)*

Table 4.22is the experimentally-derived and predicted toxicity thresholds (EC_{50}) for the senary mixtures of SDS and metal ions against*S. marcescens* (SerEW01). The experimentally-derived EC_{50S}ranged from 0.053±0.003mM (ABCR3) to 0.910±0.003mM (ABCR1) mixture ratio.In addition, only ABCR1mixture ratio was statistically different from ABCR2, ABCR3 and EECR50 mixture ratios. In all mixture ratios, the experimentally-derivedEC_{50S}, CA- and IA-predicted EC_{50}values were significantly different from oneanother ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on*S. marcescens* (SerEW01)are shown in Table 4.23. The toxic index (TI) values ranged from 0.115 ± 0.003 to 0.219±0.004, while model deviation ratio (MDR) ranged from 4.551 ±0.082 to 10.996±2.198 for CA and 8.068±0.517 to16.216±1.042. At all the senary mixture ratios tested, the metals and SDS mixtures were synergistic in their action against the bacterium. The experimental dose-response relationships of the senary mixtures as well as the predictions made on the basis of the CA and IA-models against*S. marcescens* (SerEW01) are shown in Figure 4.41. The CA and IA models greatlyunderestimated the toxicities relative to the experimentally-derived data.The mixtures equally inhibited the dehydrogenase activity at lower concentrations.

Table 4.22: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Senary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

Toxicant Senary Mixtures	EC_{50}(mM) ‡±		
	Experimental†	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)+Ni(II)			
SDS 85.53% + Cd(II) 1.96% + Zn(II) 1.12% + Pb(II) 3.76% + Co(II) 1.68% + Ni(II) 5.95% (EECR50)	0.056 ± 0.002a*	0.614 ± 0.137**	0.902 ± 0.034***
SDS 86% + Cd(II) 2% + Zn(II) 4% + Pb(II) 2% + Co(II) 3% + Ni(II) 3%(ABCR1)	0.910 ± 0.003b*	0.416 ± 0.023**	0.736 ± 0.019***
SDS 85% + Cd(II) 2% + Zn(II) 4% + Pb(II) 2% + Co(II) 3% + Ni(II) 4%(ABCR2)	0.072 ± 0.003a*	0.401 ± 0.023**	0.729 ± 0.035***
SDS 84% + Cd(II) 3% + Zn(II) 3% + Pb(II) 3% + Co(II) 5% + Ni(II) 2%(ABCR3)	0.053 ± 0.003a*	0.389 ± 0.019**	0.746 ± 0.031***

±Values are reported as Mean + 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other (P <0.05).

‡Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted EC_{50} and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other (P <0.05).

Table 4.23: Toxic Index, Model Deviation Ratio and Effect of Senary Mixtures of Metals and SDS on *S. marcescens* (SerEW01)

Metal+SDS Senary Mixtures	Toxic Index (TI) ±	MDR±		Effect
		CA	IA	
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)+Ni(II)				
SDS 85.53% +Cd (II) 1.96%+Zn(II) 1.12%+Pb(II) 3.76% +Co(II) 1.68%+Ni(II) 5.95% (EECR50)	0.115 ± 0.003	10.996±2.198	16.216±1.042	Synergistic
SDS 86% +Cd (II) 2%Zn(II) 4%+Pb(II) 2% +Co(II) 3%+ Ni(II) 3% (ABCR1)	0.219 ± 0.004	4.551 ± 0.082	8.068 ± 0.517	Synergistic
SDS 85% +Cd (II) 2%+Zn(II) 4%+Pb(II) 2% +Co(II) 3%+ Ni(II) 4% (ABCR2)	0.180 ± 0.003	5.560 ± 0.088	10.127±0.332	Synergistic
SDS 84% +Cd (II) 3%+Zn(II) 3%+Pb(II) 3% +Co(II) 5%+ Ni(II) 2% (ABCR3)	0.135 ± 0.001	7.388 ± 0.058	14.211±1.271	Synergistic

±Values are reported as Mean ± 1SD

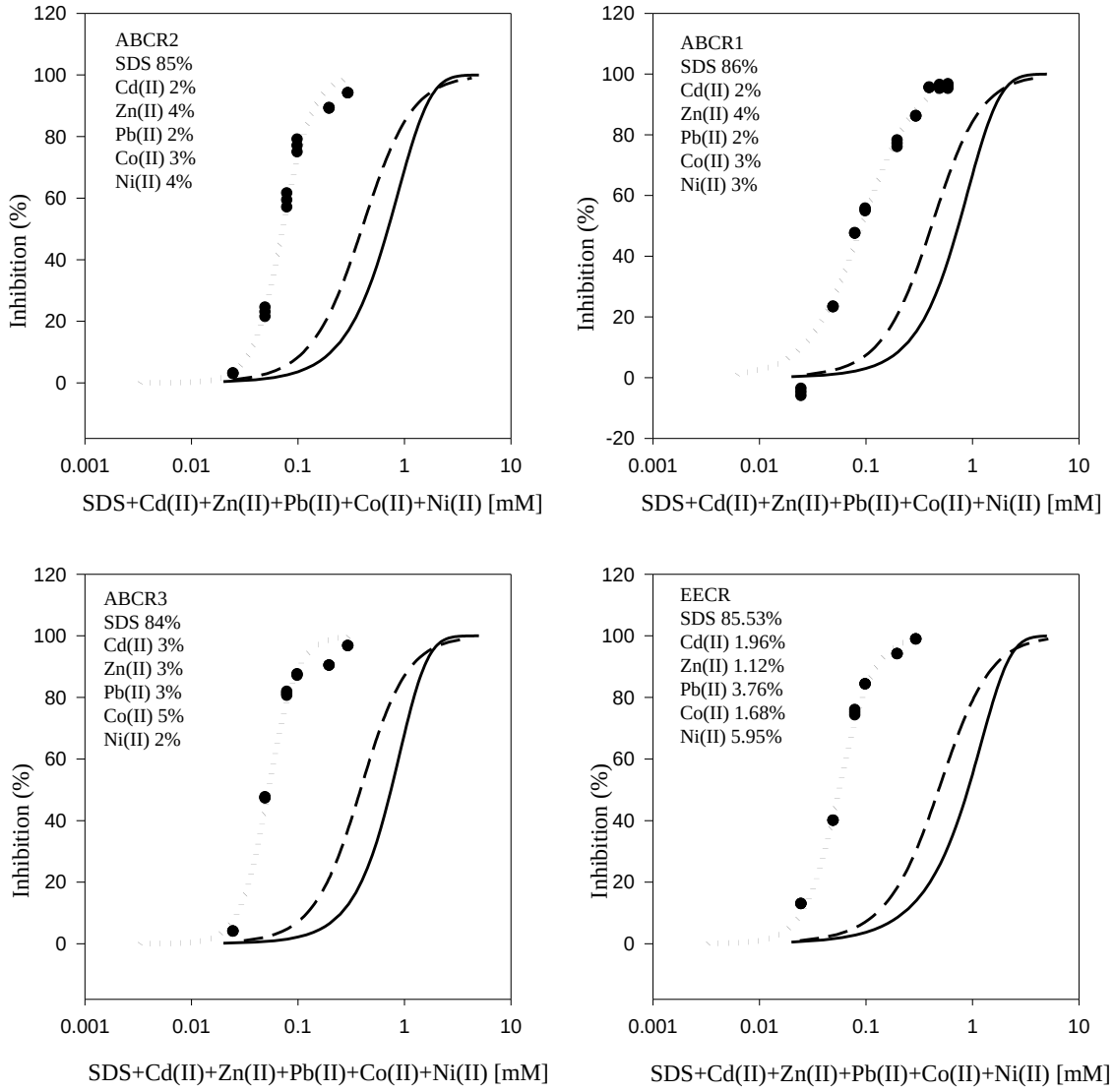


Figure 4.41: Experimental and predicted inhibitory effects of senary mixtures ofSDS, cadmium, zinc, lead cobalt and nickel ions on *S. marcescens* (SerEW01) dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

4.3.6.2.Toxicity of senary mixtures of SDS and metal ions to*A. seifertii*

Table 4.24is the experimentally-derived and predicted toxicity thresholds (EC_{50}) of senary mixtures of SDS and metal ions on *A.seifertii*. The experimentally-derived EC_{50S}range from 0.067±0.002mM (EECR50) to 0.170±0.003mM (ABCR2) mixture ratios. The experimentally-derived EC_{50S}, showed that the EECR50and ABCR1 mixture ratios were statistically different from ABCR2 and ABCR3. In all mixture ratios, the experimentally-derivedEC_{50S}, CA- and IA-predicted EC_{50S} were significantly different from oneanother ($P < 0.05$).

The toxic index, model deviation ratio and effect of metals and SDS binary mixtures on*A.seifertii* are shown in Table 4.25. The toxic index (TI) values ranged from 0.168±0.005 to 0.691±0.033, while model deviation ratio (MDR) ranged from 1.450±0.070 to 5.955±0.191 for CA and 1.694±0.074 to 7.467±0.384. In all the mixture ratios tested, the metals and SDS senary mixtures were synergistic in their action against the bacterium. The experimental dose-response relationships of the senary mixtures as well as the predictions made from CA and IA models against*A.seifertii*are shown in Figure 4.42. The CA and IA models slightly predicted higher toxicities at low concentrations while predicting lower toxicities at higher concentrations, relative to the experimentally-derived data, especially for ABCR2 and ABCR3 mixture ratios. EECR50andABCR1 mixture ratios however inhibited dehydrogenase activity in *A.seifertii*,even at low concentrations.

Table 4.24: Experimentally-Derived and Predicted Toxicity Thresholds (EC_{50}) of Senary Mixtures of Metals and SDS on *A.seifertii*

Toxicant Senary Mixtures	EC_{50} (mM) ‡		
	Experimental†	**CA-Predicted**	**IA-Predicted**
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)+Ni(II)			
SDS 88.90% + Cd(II) 1.82% + Zn(II) 1.18% + Pb(II) 3.64% + Co(II) 0.83% + Ni(II) 3.64% (EECR50)	0.067 ± 0.002a*	0.399 ± 0.024**	0.500 ± 0.056***
SDS 89% + Cd(II) 1% + Zn(II) 2% + Pb(II) 2% + Co(II) 3% + Ni(II) 3% (ABR1)	0.142 ± 0.008b*	0.421 ± 0.041**	0.551 ± 0.009***
SDS 88% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% + Co(II) 2% + Ni(II) 3% (ABR2)	0.170 ± 0.003c*	0.322 ± 0.024**	0.397 ± 0.059***
SDS 87% + Cd(II) 3% + Zn(II) 3% + Pb(II) 2% + Co(II) 3% + Ni(II) 2% (ABCR3)	0.163 ± 0.004c*	0.237 ± 0.018**	0.277 ± 0.154***

± Values are reported as Mean ± 1SD

†Within columns, in each toxicant mixture type, the experimental EC_{50}, values with the same letters are not significantly different from each other ($P < 0.05$).

‡Within rows, in each mixture ratio, comparing between the experimental EC_{50}, CA-predicted $EC_{50}$and IA-predicted EC_{50}, values with the same number of asterisks are not significantly different from each other ($P < 0.05$).

Table 4.25: Toxic Index, Model Deviation Ratio and Effect of Senary Mixtures of Metals and SDS on *A.seifertii*

Metal+SDS Senary Mixtures	Toxic Index (TI) [+]	MDR[+]		Effect
		CA	IA	
SDS+Cd(II)+Zn(II)+Pb(II)+Co(II)+Ni(II)				
SDS 88.90% + Cd(II) 1.82% + Zn(II) 1.18% + Pb(II) 3.64% + Co(II) 0.83% + Ni(II) 3.64% (EECR50)	0.168 ± 0.005	5.955 ± 0.191	7.467 ± 0.384	Synergistic
SDS 89% + Cd(II) 1% + Zn(II) 2% + Pb(II) 2% + Co(II) 3% + Ni(II) 3% (ABR1)	0.333 ± 0.015	3.011 ± 0.133	3.899 ± 0.278	Synergistic
SDS 88% + Cd(II) 2% + Zn(II) 3% + Pb(II) 2% + Co(II) 2% + Ni(II) 3% (ABR2)	0.531 ± 0.028	1.888 ± 0.101	2.329 ± 0.113	Synergistic
SDS 87% + Cd(II) 3% + Zn(II) 3% + Pb(II) 2% + Co(II) 3% + Ni(II) 2% (ABCR3)	0.691 ± 0.033	1.450 ± 0.070	1.694 ± 0.074	Synergistic

[+]Values are reported as Mean ± 1SD

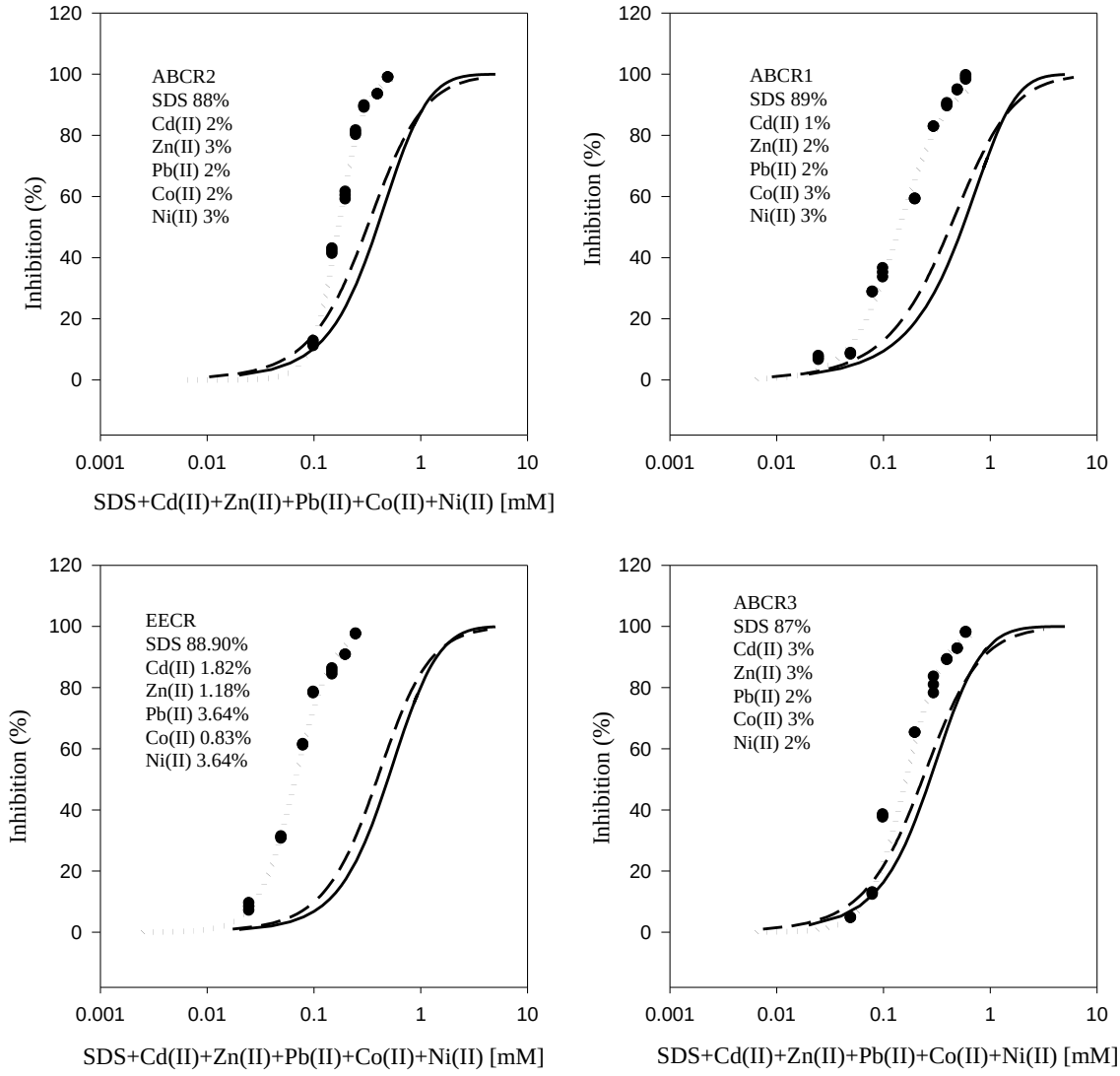


Figure 4.42: Experimental and predicted inhibitory effects of senary mixtures ofSDS, cadmium, zinc, lead cobalt and nickel ionson *A.seifertii*dehydrogenase activity.The data points represent experimental dose-response data, while dotted lines represent toxicities obtained by fitting experimental data to logistic model (Eq. 2). Dashed and solid lines represent toxicities predicted from the concentration addition and the independent action models.

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. DISCUSSION

The pH of the surface waters is important to aquatic life because pH affects the ability of fish and other organisms to regulate basic life-sustaining processes, primarily the exchanges of respiratory gases and salts with the water in which they live (Anon, 2004). The pH of the water body is also known to affect the dissolved oxygen level in fresh water. The pH of Otamiri river water was 6.42, while that of the sediment was 5.40. The pH of both the river water and sediment were slightly acidic, with the sediment being more acidic. The pH of the river water,was however, within the WHO recommended range for drinking water (WHO,2006). In addition, pH range of 6.45-7.56 has been reported for Otamiri river by previous authors (Iwuoha *et al.*,2013; Dike*et al.*,2016; Okoro*et al.*,2016). Although pH ranges of 6.31-6.60 and 6.30-6.50 have been reported for Otamiri sediment for dry and rainy seasons respectively (Iwuoha*et al.*,2012), the pH of 5.40 recorded for Otamiri sediment in this study was moderately acidic. This could be attributed to increasing pollution of the river through dumping of untreated wastes and leachates from solid wastes (Iwuoha *et al*., 2013).

The temperature of the river was 26.1^{o}C, this is below the WHO recommended range of 27-28^{o}C (WHO, 2006). Average temperature range of 26.9-28^{o}C has, however, been reported for Otamiri river in previous studies (Dike *et al*., 2016: Okoro *et al*., 2016). Temperature is known to affect the dissolved oxygen level in aquatic ecosystem, which in turn has deleterious effects on various aquatic biotas there-in. The high phosphate recorded in Otamiri sediment in this study could be traced to agricultural and industrial sources (Okoro *et al*., 2016). Such high levels of phosphates could lead to excessive proliferation of algae (algal bloom), with its

resultant effects on dissolved oxygen level in the river. However, this high level of phosphate in the sediment was not replicated in the river water (0.032 Mg/l). Such low levels of phosphates have been reported for the river by previous authors (Dike *et al*., 2016;Okoro *et al*., 2016). Similarly, phosphate range of 0.8-5.6 Mg/l, which is above the WHO recommended standard for drinking water, has been reported for Otamiri (Okeke and Adinna, 2013).The BOD was of the same value as the World Health Organization recommended standard while Dissolved oxygen (DO) recorded in this study was just slightly below the WHO recommended standard for drinking water (WHO, 2006). The relatively low levels of BOD and DO in the present study is surprising, as dumping of both degradable and non-degradable wastes in Otamiri has been reported to gradually becoming a norm rather than an exception (Temitope*et al.*,2016). The high turbidity recorded in this study relative to the WHO recommended standard for drinking water could be attributed to run-off from farm lands at the bank of the river, prolonged human activities in the river as well as increasing sand mining or dredging going on in the river (Temitope*et al.,* 2016). Similarly, the high levels of electrical conductivity observed in the study could be due to the high content of major ions in Otamiri river water, as reported by Okeke and Adinna, (2013) and Okoro *et al*. (2016).

Heavy metals in waters and sediment could result from the weathering of parent rocksor anthropogenic activities. In recent past, so many studies have been undertaken on the heavy metals contents of Otamiri river and sediment (Iwuoha *et al*., 2012: Iwuoha *et al*., 2013; Okeke and Adinna, 2013; Temitope*et al.*, 2016; Onyekuru*et al.*,2017). Some of these studies showed that there has been a progressive accumulation of heavy metals in Otamiri sediment and by extension the river as result of industrial, agricultural or domestic activities. Among the heavy metals studied, lead, cadmium, nickel and mercury contents of Otamiri river were above the

WHO recommended standards for drinking water (WHO, 2006). Generally, the high levels of these heavy metals in the river could be as a result of indiscriminate dumping and subsequent burning of such solid wastes at the river bank, activities at auto-mechanic workshops at Nekede mechanic village, run-offs from Owerri urban and environs, unrestricted discharging of untreated industrial and domestic effluent into the river. In addition, the slight acidic pH recorded in the river might have also contributed to the high levels of some of these metals, as low pH has been shown to enhance the release of heavy metals from polluted sediment (Zhang *et al*., 2018). The 0.066 and 1.054 mg/L concentrations of nickel observed for Otamiri river and sediment respectively in this study were higher than the concentration range of between 0.005 and 0.010mg/L of dissolved nickel generally reported in aquatic ecosystems (Galvin, 1996). The toxicity of nickel to aquatic life has been shown to vary significantly with species of organisms, pH and water hardness (Birge and Black, 1980; Dallas and Day, 1993). Nickel toxicity is generally low but elevated concentrations have been reported to cause sub lethal effects (Khangarot and Ray, 1990). The relatively high level of iron over other heavy metals in both river water and sediment attributed to the high level of iron in the upper earth crust of southern Nigeria (Iwuoha *et al.,* 2012). The absence of cobalt and reduced levels of some of the heavy metals in the river water could be attributed to their inability to remain in solution. Nweke*et al.*,(2006), made similar observation in New Calabar river. In addition, the occurrence of higher amount of cadmium in the river water as against the sediment could be attributed to the physical disturbances, such as sand dredging/mining going-on in the river.

The heavy metals content of Otamiri sediment was higher than those of the river water. This could be attributed to the accumulative nature of heavy metals in the sediment. According to Hanson*et al*.,(1993), metals bind to organic and inorganic particles that eventually settle to the

bottom of streams, rivers, reservoirs, lakes, estuaries or marine waters. This observation is in agreement with the previous report that in an undisturbed aquatic environment, metals are preferentially transferred from the river phase to the sediment and thus metal concentrations in sediment are generally much higher than in the overlying water (Bryan and Langston, 1992).

In Nigeria, reports on surfactants levels in surface waters are very scarce and Otamiri river is not an exception. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been reported to be ubiquitous in river water, oceans, sediments, soil and tissues of wildlife and humans (Giesy and Kannan, 2001; Higgins *et al.*,2005;Ahrens*et al.*,2010a;Wang*et al.*,2013). In this study, perfluorobutanesulfate (PFBS) was not detected in Otamiri river water. Although at present, perfluorinated surfactants and their precursors are not included in regular quality controls neither of surface waters nor drinking water or organic waste materials.Skutlarck*et al.*(2006), in their study on perfluorinated surfactants in surface and drinking waters, recorded PFBS ranges of 0-46ng/L, 0-1450ng/L and 0-71ng/L respectively for Rhine, Moehne and Ruhr rivers and their selected tributaries. Similarly, Saito *et al*. (2004), recorded lowest limits of detection of 0.06 and 0.04 ng/L for perfluorooctane aceate (PFOA) and perfluorooctane sulfonate(PFOS) respectively in their study on perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. The non detection of PFBS in Otamiri riverwater could probably be attributed to its concentration being below the detectable limit of the equipment used or the method applied. Otamiri sediment however recorded 0.0142mg/kg of PFBS.

Sediment is an important sink and reservoir of persistent organic pollutants and has a large impact on their distribution, transportation, and fate in the aquatic environment (Ahrens *et al.*, 2009; Yang*et al.*,2011). The distribution of PFAS between water and sediment is considered as an important process which controls their transport and fate (Prevedouros*et al.*,2006; You*et*

al.,2010). Sediment-water distribution is a complex process, depending not only on the physicochemical characteristics of the compounds but also on the sediment nature such as the organic carbon fraction (Liu and Lee 2005; Higgins and Luthy, 2006; Ahrens *et al*., 2010b; Zhao *et al*., 2012). The slightly acidic pH of Otamiri river water as observed in this study (5.40) may have also contributed to the detection of PFBS in Otamiri sediment but not in the river water. Studies under laboratory conditions showed that adsorption of PFAS were generally greater with decreasing pH of the water and increasing organic carbon fractions of sediments (Higgins and Luthy, 2006).

Ammonium lauryl sulfate was one of the anionic surfactants detected in both Otamiri river and sediment at 0.070 mg/l and 0.0303mg/kg respectively. Their level of distribution in water and sediment in this study could be due to its high solubility. Though ammonium lauryl sulfate itself is not toxic, it is a nitrosating agent. Nitrosating agents may decompose and or react to cause nitrosamine contamination. Once in the body, nitrosamines are activated by cytochrome P-140 enzymes, they are believed to induce their carcinogenic effects by forming DNA adducts at the N-and O-atoms (Oyama *et al*., 2007; Sasaki *et al*., 2008).

Sodium methyl sulfate was detected both in Otamiri river and sediment. The proportion found in the river water (0.060mg/l) was slightly higher than in the sediment (0.0532mg/l). This could be attributed to its high solubility. In addition, anionic surfactants are known to form foam; such stable foam formation in the river is highly undesirable because it blocks the transformation of the oxygen-mass from air to water. Hydrophilic constituents of toxic surfactants can endanger the survival of aquatic animals and bacteria in water (Effendi *et al*., 2017).

Sodium dodecyl sulfate (SDS) is not currently monitored in water systems or listed as a ground water contaminant (Kegley *et al.,* 2014). SDS was relatively high in Otamiri river water (0.10 mg/l) and sediment (0.4531 mg/l). In this study, both the river water and sediment had concentrations of SDS higher than the permissible limit (0.02 mg/l) for anionic surfactant in class I water (Pastewski and Medrzycka, 2003). Similarly, the sediment recordedconcentrations higher than the non-effect concentration value (0.25 mg/l) for surfactants as reported by Van de Plassche *et al*. (1999).In addition, anionic LAS have been reported to be preferentially adsorbed to sediments (Sanderson *et al.,* 2006).Similarly, in a study on aquatic environmental monitoring and removal efficiency of detergents, LAS variations between surface and bottom waters were reported (Abd El-Gawad, 2014).

In addition, surfactant concentrations in surface waters as high as 0.416 mg/l has been recorded in the United Kingdom (Fox *et al*., 2000). In Massachusetts, the Town River had reported concentrations between 0.04 and 0.590 mg/l, while other major rivers in the United States had reported 0.01 to 3.30 mg/l or 0.01 to 0.04 mg/l (A.D. Little Co. 1981;Lewis and Wee, 1983; Hennes and Rapaport, 1989). Nevertheless, these levels of SDS in Otamiri river and sediment could pose a great danger to aquatic lives in the river.The anionic surfactant Sodium laureth sulfate was also detected in the river and sediment. Though ecotoxicity studies have determined that a surfactant concentration of 0.5 mg/l in natural water could be essentially nontoxic to fish and other aquatic life under most conditions (Abel, 1974), it is however suggested that chronic toxicity of anionic surfactants occur at concentrations as low as 0.1 mg/l (Lewis, 1991).Furthermore, surfactants have been reported to combine with heavy metal ions thus enhancing the toxicity of heavy metals to fishes and other aquatic organisms (Karbe, 1975; Swedmark*et al.*,1978).

The higher concentration of most of these anionic surfactants in the river phase compared to the sediment fraction in this study could be attributed to the continuous sand dredging going-on in the river, as it has been previously reported that physical disturbances could cause the re-distribution of the sediment-associated contaminants in the water phase to disturb the activities of suspended microorganisms (Nweke and Orji, 2009). Generally, the presence of these anionic surfactants in Otamiri river and sediment might be attributed to unrestricted discharge of untreated domestic sewage from Owerri urban and environs, laundry and car washing outfits located at the bank of the river among others.

The bacteria isolated from Otamiri river include: *Serratia marcescens* (SerEW01), *Staphylococcus*sp, *Streptococcus*sp, *Enterococcus* sp and *Escherichia coli*. In the sediment however, *Streptococcus*sp, *Pseudomonas*sp, *Klebsiella* sp, *Acinetobacterseifertii*, *Bacillus*spand *Escherichia coli* were isolated. These isolates have been reported by previous authors that have worked in the river and its sediment (; Ogbulie *et al.*,2010; Ogah *et al.*,2018;Fagorite *et al*., 2019).

Among the isolates from the river,*S. marcescens*(SerEW01)recorded the highest percentage occurrence of 33.33%, followed by *Staphylococcus* and *Streptococcus* species (22.2%). These are opportunistic pathogens of human origin (Greenwood *et al.*,1992).*Serratiamarcescens* (SerEW01) is known to cause hospital-acquired infections, particularly catheter-associated bacteremia, urinary and respiratory tract, as well as wound infections (Greenwood*et al.*,1992).The presence of *Streptococci*sp and *Escherichiacoli* was an indication of the poor sanitary quality of the Otamiri river water (Ibeneme *et al*., 2014).World Health Organization recommended one *E. coli* cell per 100 ml of water sample to be normal (WHO, 2006).

However, these indicator organisms, as well as *Klebsiella* species were found in large numbers in Otamiri river water and sediment, indicating possible feacal contamination.

In the Otamiri sediment,*Pseudomonas*and *Bacillus*species were fairly prevalent. This could be attributed to their wide spread in water and soil ecosystems as reported by Roggers*et al.*,(1977). *A.seifertii* recorded the highest percentage occurrence of 42.10%. The presence of hospitals and other medical facilities near the banks of at the bank of Nworie river (a tributary of Otamiri) may have contributed to the high percentage occurrences of some of these human pathogens in the river and its sediment. In addition, some anionic surfactants were prevalent in the river and sediment as shown in Table 4.1, the presence of such substrates can stimulate the proliferation of such organisms that can utilize them as carbon and energy sources. Some of these isolates have been reported to degrade anionic surfactants and other detergents (Ogbulie *et al*., 2010; Anaukwu *et al*., 2016;Abimbola and Iyanuoluwa,2017;Abimbola*et al.*,2018).

Heavymetal contamination of aquatic environment has been a serious issue because of their persistence and toxicity (Lee *et al.*,2005). Apart from natural sources, heavy metals are deposited into the aquatic ecosystems from myriad of industrial activities. Cadmium, cobalt, nickel and zinc have many industrial applications and thus co-contaminate soil and aquatic habitats (Nies, 1992). Similarly, Sodium dodecyl sulfate (SDS) is the most widely used synthetic organic chemical found in detergents, shampoos, cosmetics, herbicides, household cleaners among others (Cowan-Ellsberry *et al.,* 2014). Different microbial responses have been used to assess toxicity of xenobiotic chemicals to microorganisms. Among these responses is the dehydrogenase activity of the microorganisms. Microbial dehydrogenases are intracellular, rapidly degraded after cell death and are common to all microorganisms (Rossel and Tarradellas, 1991). Thus, their activity could be used to evaluate toxicity of xenobiotics to

microbial viability. Dehydrogenase activity has been used to assess the toxicity of chemical compounds to pure cultures and microbial community (Nweke *et al.*,2015; 2016; 2017; 2018). In the present study, *S. marcescens* (SerEW01)appears to be generally more sensitive to most of the heavy metals and SDS tested than *A. seifertii*, except for cadmium and cobalt. This observation however is in line with the numerous reports that planktonic bacteria are more sensitive to aquatic pollution than their sediment counterparts (Hornor and Hilt, 1985; Silver and Misra, 1988; Romero *et al.,*1999; Nweke *et al.,* 2007a). Similarly, apart from cadmium, the concentrations of the other toxicants studied in the present research were higher in Otamiri sediment than in the river water, lending credence to the report that heavy metals discharged into estuarine and coastal waters rapidily become associated with particulates and are incorporated in bottom sediments (Hanson *et al*., 1993). The relative tolerance of *S. marcescens* (SerEW01)to cobalt, and cadmium compared to *A. seifertii*, even when cobalt was not detected in the river water, as recorded in this study could not be understood. *Serratia*tolerance to zinc and other heavy metals has however been reported (Cider*et al.*,2017; Nwagwu*et al.*,2017). Similarly, better tolerance to heavy metal toxicity by Gram negative compared to Gram positive bacteria has also been reported (Morozzi *et al.*,1986;Minz*et al.*,1996; Nweke *et al*., 2007a). In the present study, cobalt inhibited dehydrogenase activity of *S.marcescens* (SerEW01) and *A. seifertii* even at low concentrations. Nickel and cobalt toxicity to microorganisms have been widely reported and have been critically reviewed by Gikas (2008). Cobolt has also been reported to be more potent growth inhibitor to microorganisms than nickel (Chandy, 1999; Gikas, 2007;Nweke *et al*., 2018). The same trend was observed in this study for both planktonic and sediment bacteria.According to Hashida and Inouye (2007), increase in cobalt concentrations to 2 mM stimulated (increase) thermolysin (from *Bacillus*

thermoproteolyticus) activity 3 to 4 times but this enhanced activity was considerably reduced with higher cobalt concentration (2-18 mM).

Nickel has also been reported to stimulate microbial growth at concentrations approximately below 27 mg/L (≈ 0.46 mM), in a study on the kinetic response of activated sludge to individual and joint nickel (Ni(II)) and cobalt (Co(II)) (Gikas, 2007).In the present study, both *S.marcescens* (SerEW01) and *A. seifertii*recorded similar EC_{50S} for nickel, 0.100 ± 0,008 and 0.649 ± 0.053 mM respectively. Similar report was recorded for nickel in a study on the microbial community of New Calabar River by Nweke and Orji, (2009).They also recorded EC_{50S} of 2.47 and < 6 mM Ni(II) for planktonic and sediment populations respectively in the same study.

Although cobalt, nickel and zinc are trace elements, they can be toxic to bacteria at high concentrations. This is in line with their observed toxicities in the present study. For instance, zinc is a component of many microbial enzymes, where it is necessary for their catalytic functions and structural stability (Choudhury and Srivastava, 2001). However, Zn(II) can become toxic to cells at high concentrations. Zinc for example is known to be inhibitory to respiratory electron transport system of bacteria and eukaryotic organisms (Kashara and Anraku, 1974; Beard *et al.*,1995;Nweke and Orji, 2009). Zinc inhibited dehydrogenase activity by 50% in sediment bacteria from New Calabar River at 0.166 and 0.873 mM for *Bacillus* and *Micrococcus* species respectively (Nweke *et al*., 2007a).Similarly**,** EC_{50}range of 0.236 ± 0.044 to 0.864 ± 0.138 mMforzinc was reported for planktonic bacteria of New Calaba River byNwewke *et al*. (2006).In the present study however, EC_{50S} of 0.046 ± 0.003 and 0.075 ± 0.005 mM Zn(II) were recorded for zinc, against *S. marcescens* (SerEW01)and *A. seifertii* respectively.The higher toxicity of zinc to *S. marcescens* (SerEW01)compared to cadmium, as

well as relative tolerance to lead by this bacterium as recorded in this study is not quite understood. Nevertheless, high tolerance of *S.marcescens* to lead and cadmium has been reported (Cristani *et al.,* 2011).

S. marcescens (SerEW01) was more tolerant to cadmium than *A. seifertii* as observed in the present study. This could be attributed to the former's adaptation to the relatively higher cadmium concentrationsin Otamiri river water over time, as the heavy metal recorded relatively higher concentration in the river water compared tothe sediment.Cadmium and lead have no known physiological functions and have been reported to be generally more toxic than the trace elements(Nies, 1999).Cadmium displaces Ca(II) and Zn(II) in proteins and cause oxidative stress(Stohs and Bagchi, 1995;Goyer, 1997). Furthermore, Cd(II) could disrupt the integrity of microbial cell membrane and disturb the proton flux through the membrane (Bitton*et al.,*1988). An IC_{50} ranging from 0.199 mM to 0.239 mM Cd(II) against bioluminescence in photobacterium Q67 was reported by Ge,*et al.*,(2014). Similarly, IC_{50} of 0.022 mM Cd(II) against *Pseudomonasfluorescens* was reported by Nweke *et al.* (2018). However, in the present study, median inhibitory concentrations of 0.058 ± 0.002 and 0.113 ± 0.005 mM were recorded for cadmium and lead respectively. Maximum tolerance concentration of 4 mg/ml Pb(II) (≈ $2.0x10^{-5}$ mM) and 1.5mg/ml Cd(II) (≈ $1.3x10^{-5}$ mM) were reported for *S.marcescens* from a tropical stream by Nwagwu *et al.* (2017). Furthermore, EC_{50}s of 0.011 ± 0.000 and 0.222 ± 0.005 mM respectively were observed for cadmium and lead in the present syudy against *A. seifertii*. Cadmium was thus the most toxic against*A. seifertii*, from the sediment among the studied toxicants as individuals. Such high toxicity of cadmium to sediment bacterial population,with an EC_{50}< 0.2 mM has been reported(Nweke and Orji, 2009). Lead was reported

to have detectable effects upon soil microbial community diversity, even at 1ppm (≈ 0.005 mM) (Sobolev and Begonia, 2008).

Information on the effects of SDS on microbial dehydrogenase activityis scarce. However, toxicity of SDS to algae and aquaticmacrobiota, using other responses has been reported. Guilherminoet *al.*(2000), in their study on*invitro* and *invivo* inhibition of *Daphnia magna*acetyl cholinesterase by surfactant agents (dodecyl benzyl sulfonate (DBS), sodium dodecyl sulfate (SDS), and a domestic detergent (Y)) reported an EC_{50} of 51.5 mg/L (≈ 0.18 mM). In the present study, the planktonic bacterium *S.marcescens* (SerEW01) with an EC_{50} of 2.329 ± 0.092 mM was relatively more sensitive to SDS than the sediment bacterium *A. seifertii*(EC_{50} of 2.810 ± 0.140 Mm). In a study consisting of various taxa, an EC_{50}value of 2.6 mg/L SDS (≈ 9.02 x 10^{-3}mM SDS), was reported for the bacterium *Vibrio fischeri* (Mariani *et al*., 2006).

SDS has also been reported to inhibit both growth and rate ofphosphrus uptake in pure culture of *Acinetobacter junii* by 100% at concentrations of 10^{-3} mol l^{-1} (1 mM) and higher, with an EC_{50} of 5.00 ± 2.95 x 10^{-6}mol l^{-1} (0.005 mM) and 3.33 ± 0.96 x 10^{-4} mol l^{-1} (0.33 mM) respectively (Hrenovic and Ivankovic, 2007). Similarly, sewage sludge isolates, *A. johnsonii* and Oligotropha carboxidovorans showed nearly 20% and 50% loss of viability during the treatment with 0.2 and 2 mg ml^{-1}SDS (≈ 6.94 x 10^{-1} and ≈ 6.94 mM), respectively (Malik *et al*., 2005). Furthermore, toxicity of SDS to luminescent bacterium (*Photobacterium phosphoreum),* unicellular alga (*Scenedesmusquadricauda*), protozoan (*Parameciumcaudatum*) and crustacean (*Daphniamagna*) has been reported (Evsyunina *et al*., 2016).

The order of toxicants decreasing toxicities as recorded in the present studyis Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS and Cd(II) > Co(II) > Zn(II) >Pb(II) > Ni(II) > SDS, against*S.marcescens*and *A. seifertii* respectively. Stimulatory effects have been reported for

many microbial processes for metal ions, including dehydrogenase activity, growth and bioluminescence (Visca *et al.,* 1992; Christofi*et al.*,2002;Osman *et al.*,2004; Gikas, 2007;Rodea-Palomares *et al.*,2009; Nweke *et al.,* 2018) and for SDS (Tozum-Calgen and Atay-Guneyman, 1994;Dirilgen and Ince, 1995). However, the absence of stimulatory effect by the individual metals and SDS in the present study could be due to the sensitivity of both bacteria to the effects toxicants.In addition, the shapes of the dose-response curves are rather similar for some of the toxicants, suggesting possible similarity in the molecular mechanism of actions of some of the toxicants.

In aquatic environment, microorganisms are exposed to mixture of chemicals whose toxicity is different from those of their individual components. These chemicals may also interact to modulate the toxicity of each other in the mixture.This has been established in this study with SDS and each of the five heavy metals against planktonic and sediment bacteria.SDS modulated the toxicity of the heavy metals and vice versa, giving EC_{50S} higher than those of the individual heavy metals but lower than that of SDS, in all the binary mixtures tested for both bacteria. This modulation however seems to be dependent on the relative proportions of the most toxic (heavy metals) and least toxic component (SDS) present in the mixture. Similarobservation was made by Nweke *et al*. (2014).

In a study on the effects of mixtures of heavy metals and a surfactant on the development of cod (*Gadus morhua* L), Swedmark and Granmo (1981) also reported differences in toxicity between the combinations of metals and surfactant (LAS) and their single components. They also noted that generally, the surfactant decreased the toxicity of copper, while zinc decreased that of LAS. These metals ions may have been partially stabilized by SDS through either complexation or counter ion exchange with the surfactant (Friedel *et al.*,1994; Juang*et al.*,2003;

Masakorala*et al.*,2008). These could result in reduction in the amount of heavy metals and SDS to which the bacteria were exposed to.The observed toxicity thresholds (EC_{50S}) of the binary mixtures showed that at all effect concentrations, *S. marcescens* (SerEW01) are generally more sensitive to the toxic effects of SDS and metal ions than *A seifertii*.In the present study, apart from cadmium, the concentrations of other toxicants were higher in the sediment compared to the river water. Organisms isolated heavy metals polluted habitats are more tolerant to metals than organisms isolated from unpolluted habitats. Our result agrees with this assertion.

The isobolographic analysis based on the EC_{50S}, model deviation ratios (MDR) and the toxic index model (TI) used to analyse binary mixture toxicity indicated similar effects, with regards to the toxicity of SDS+metal mixtures against the dehydrogenase activity of *S. marcescens* (SerEW01) and *A seifertii*.According to Boillot and Perrodim (2008), TI = 1, describes additive interaction, TI > 1 describes antagonistic interaction, while TI < 1 describes synergistic interaction. Similarly, MDR values < 0.5 describes antagonism, > 2 describes synergism while MDR values of $0.5 \leq MDR \geq 2$ describes additivity (Cedergreen, 2014).The TI values for all the binary mixtures for *S. marcescens* (SerEW01) were less than 1, thus describing synergistic interactions. Synergistic interactions have been reported for the toxicity of binary mixtures of heavy metals and organic compounds to microbial species (Nweke *et al.*, 2014; 2015; Lin*et al.*,2016;Cai *et al.*, 2019).

The MDR and TI values for *A. seifertii*showed that SDS 96% + Cd(II) 4% mixture ratio was antagonistic, while ABCR1 and ABCR2 mixture ratios of SDS + Cd(II) showed weak synergistic interactions. In addition, other binary mixtures were synergistic in their action. Antagonistic effects have reported for joint toxicity of LAS and heavy metals against cod (*Gadus morhua* L), as well as LAS and anthracene on the growth of a microbial consortium

isolated from polluted sediment (Swedmark and Granmo, 1981; Flores *et al*., 2010). Similarly, the weak synergistic effect observed in the present study could be attributed to the masking effect of SDS on cadmium ions in the mixture. Similar observation was reported by Nweke *et al*. (2014), on the toxicity of binary mixtures of formulated glyphosate and phenols to *Rhizobium* species. In addition, weak synergistic interactions at higher concentrations (≥ 1 + 5 µ/ml)of MCLR and LAS on toxin bioaccumulation in duckweed (*Lemna minor*) were also reported byWang*et al.*(2012). Furthermore, weak synergistic effects were also reported for the joint toxicity of perfluorooctane sulfonate/perfluorooctanoic acid (PFOS/PFOA) and copper to *Carassius auratus*and between copper and perfluorinated carboxylic acids (PFCAs) (Feng *et al*., 2015; Cai *et al.,* 2019). These differences could be attributed to differences in the chemical structure of the surfactants and the organizational levels of the organisms. However, other researchers have reported joint toxicity of LAS and heavy metals, as well as LAS and anthracene to be antagonistic against cod (*Gadus morhua*L)and on the growth of a microbial consortium isolated from polluted sediment (Swedmark and Granmo, 1981; Flores *et al.,* 2010). CA and IA models have been used to predict the toxicity of chemical mixtures based on the concentration-response relationship of the components of the mixture. The CA model is based on the assumption that the components of the mixture acts similarly, while IA model assumes that the components of the mixture act dissimilarly. The CA and IA models were used to predict the joint action of the binary mixtures to both the planktonic (*S. marcescens* (SerEW01)) and sediment (*A. seifertii*) bacteria. In SDS 92% + Ni(II) 8% and SDS 91% + Ni(II) 9% mixture ratios for*S. marcescens* (SerEW01), both models over estimated the toxicity at low concentrations while underestimating at high concentrations. Similarly, in ABCR1 and ABCR2 mixture ratios of SDS+Ni(II), as well as all but ABCR1 mixture ratio of SDS+Co(II)

binary mixtures for *A. seifertii*, both CA and IA models overestimated the toxicity of the mixtures at low concentration, while underestimating at high concentration.These observations contradictedthe reportsby other authors on the toxicity of binary mixtures of heavy metals. For instance, using isobolographic representation, Gikas (2007), reported synergistic toxicity of binary mixtures of Ni(II) and Co(II) against growth of activated sludge microbial community. However, in the same study, Ni(II) and Co(II) mixture was antagonistic at the zone of decreasing stimulation. Similarly, Nweke *et al*. (2018), reported that both CA and IA models underestimated toxicity of a specific mixture ratio at low doses and overestimated toxicity at high doses of metal mixtures to *Pseudomonas fluorescens*. These observations indicated that the overall effect of metal ion mixture may vary with the threshold under consideration. Similar assertion could be made on the present study, despite the variations in bacteria and the toxicants studied. However, SDS 98.08% + Co(II) 1.92% mixture ratio for*S. marcescens* (SerEW01) was stimulatory at low concentration and inhibitory at high concentration of the binary mixture. This observation could probably be attributed to the organism's use of SDS as an energy or carbon source, at low concentration.

Similarly, cobalt is a co-factor in microbial enzyme systems but has been reported to be toxic to microorganisms at high concentratios. Gikas (2007), reported that all the three tested Ni(II) and Co(II) mixture ratios stimulated the growth of the activated sludge microbial communitymore drastically at relatively small concentrations, compared with the stimulation of equal concentration of single species, whilst they also acted as more potent inhibitors at relatively high concentrations.In addition, in SDS+Ni(II) mixtures for *S. marcescens* (SerEW01), as well as SDS+Ni(II), SDS+Co(II) and ABCR3 mixture ratio of SDS+Cd(II)binary mixtures for *A. seifertii*, both CA and IA models predicted identical toxicities of the binary mixture. Studies

have shown that under certain conditions, the toxicity thresholds (EC_x values) predicted by both models may be identical (Boedeker*et al.*,1993; Drescher and Boedeker, 1995; Backhaus *et al*., 2004;Zhang*et al.*,2008; Huang, 2011**)**. According to Chen *et al.* (2013), equal predictions can be produced by CA and IA models when the dose-response relationship of every individual mixture component can be described by two-parameter Weibull function, the curves are strictly parallel and the slope parameter β equals 2.3. Depending on the slope of the individual dose-response relationships, both CA and IA may produce identical prediction (Drescher and Boedeker, 1995).

According to Cedergreen*et al.*,(2007); Cedergreen *et al*., (2008), binary mixtures of chemicals that have concentration-response curves with log-logistic slope of about 1 have similar IA and CA predictions. This appears to be the case with *A. seifertii*. with the present study. The logistic function slope parameter for SDS, Co(II), Ni(II) and Cd(II) were 2.1, 0.96, 0.95 and 1.8 respectively. These values are not far from 1 and probably were the reasons for the similar CA and IA predictions observed in SDS + Ni(II), SDS + Co(II) and SDS + Cd(II) binary mixtures.Barata *et al*. (2006), reported similar predictions by CA and IA model for binary mixtures of metals and pyrethroid insecticides against *Daphnia magna.*

In SDS + Zn(II) mixtures for *S. marcescens* (SerEW01), both CA and IA models predicted slightly lower toxicities in the various mixture ratios tested.However,SDS and zinc binary mixtures showed biphasic effects upon exposure to *A. seifertii*. Biphasic response to chemicals is a phenomenon widely reported in microorganisms and higher forms of life (Calabrese and Blain, 2005). Stimulation of dehydrogenase activity at low concentrations (hormesis) and inhibition at high concentrations observed in this study is in line with the reported hormetic effects of zinc and SDS on microorganisms (Nweke *et al*., 2007a;Tozum-Calgan and Atay-

Guneyman, 1994). Furthermore, in other SDS + metalions binary mixturesboth CA and IA models grossly underestimated the toxicity of the mixtures to both planktonic and sediment bacteria.

Although SDS and metal ions may have similar modes of action against bacteria, significant difference did not exist between predicted values of mixture toxicity on the bases of CA and IA for SDS + Ni(II) and SDS + Co(II) binary mixtures, as well as SDS 96% + Cd(II) 4% mixture ratio for *A. seifertii*. Similar insignificant differences between mixture toxicity predicted on the basis of CA and IA for phenolic compounds with similar and dissimilar mechanisms of action was reported by Huang *et al*. (2011). In addition, virtually identical toxicities predicted from CA and IA models for mixtures of similar-acting phenylurea derivatives were reported by Backhaus *et al*.(2004). This shows that both concepts, concentration addition and independent action, may serve as veritable tools for predicting toxicity of chemical mixtures (Faust *et al*., 2000).

Acinetobacter seifertii was more tolerant to the ternary mixtures of the toxicants than *Serratia marcescens* (SerEW01)in the present study. However, both planktonic and sediment bacteria were more sensitive to the ternary mixtures of SDS and metal ions than the binary mixtures, as the ternary mixtures were generally more toxic than the binary mixtures of the toxicants. This is reflected in their observed toxicity thresholds(EC_{50S}). This shows possible interaction of the chemicals, resulting in modulation of the toxicity of the toxicants in the mixture. SDS modulated the toxicities of the heavy metals and vice versa, as SDS has been reported to be less toxic than a variety of metals and non surfactant compounds (Whitton, 1967; Blanck*et al.*,1984;Wangberg and Blanck, 1988). Similar differences in toxicities between binary and ternary mixtures of same toxicants have been reported (Boltes*et al.*,2012).

In the ternary mixtures against*A. seifertii,* high toxicity of cadmium ions to the organism as recoreded in the singles seems to be a major factor in the toxicity of the ternary mixtures that contain the heavy metal. Ternary mixtures that contain cadmium were the most toxic to the bacterium. This trend was however not observed in the binary mixtures. Similar trend was observed for *S. marcescens* (SerEW01) in ternary mixtures that contain zinc. Results from the individual toxicants showed that the planktonic bacterium was most sensitive to zinc as a single toxicant.Such change in the type of toxicological interactions between ternary and binary mixtures was reported by Boltes *et al*. (2012).

The model deviation ratios (MDR) and the toxic index model (TI) used to analyse the ternary mixture toxicityof SDS and metal mixtures against the dehydrogenase activities of*S. marcescens* (SerEW01) and *A. seifertii* indicated similar results, The TI and MDR indicated synergistic interactions for *S. marcescens* (SerEW01), in all ternary mixtures, while indicating both synergistic and additive interactions for *A. seifertii,* though the planktonic bacterium (*S. marcescens* (SerEW01)) showed stronger synergism.In the present study, ternary mixtures also presented higher synergism than the binary mixtures over the entire effect level for both bacteria. Some authors have reported both synergistic and antagonistic interactions in studies with ternary mixtures of heavy metals to bacteria and liver cells (Lin *et al*., 2016; Nweke *et al*., 2018). Similarly, Franklin*et al.*(2002), reported antagonism on the interactive effect of ternary mixtures copper, cadmium and zinc on metal cell binding and uptake to the alga *Chlorella* sp. Furthermore, partly additive effect was reported on the combined toxicity of cadmium, copper and lead from industrial wastewater on *Photobacterium phosphoreum*T3S by Zeb *et al*. (2016). Although the mixtures in those studies had some similar components (heavy metals) as the present study, none had SDS as a component. This may partly explain the synergistic

interactions recorded all through for *S. marcescens* (SerEW01) in the mixtures studied. Similarly, Di Poi*et al.*,(2018), reported both synergistic and antagonistic interactions in a study on toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Furthermore, the ternary mixture of chlorinated pollutants withperfluorooctane sulfonic acid(PFOS) showed very strong synergism for all effect levels (CI < 0.1). In the same study, the ternary mixture perfluorooctane sulfonic acid+ bizafibrate + gemfibrozil presented a lower antagonism than the binary mixtures of the same compounds over the entire effect level range, with CI values essentially constant (Boltes *et al.*, 2012). It has been reported that the types of interactions exhibited by the components of mixtures largely depend on the proportion of the occurrence inthe mixtures (Otitoloju, 2005).

Concentration addition and independent action models have been used to predict toxicity of chemical mixtures based on the concentration-response relationship of the components of the mixture. In all mixture ratios, both models grossly underestimated the toxic interactions of the toxicants against *S. marcescens* (SerEW01). Similarly, Nweke *et al*. (2018) reported both underestimation and overestimation of toxicity of ternary mixtures of heavy metal to *Pseudomonasfluorescens*.However, the CA and IA models either slightly or grossly underestimated the joint toxicity of the SDS and metal mixtures to *A. seifertii*. In addition, both models also made good predictions for ABCR1 mixture ratio of SDS + Ni(II) + Cd(II) ternary mixture for *A. seifertii*. Similarobservation was made by Nweke *et al*. (2018).

It is important to note that therewas no statistical difference between the experimentally-derived and CA-predicted EC_{50S}, in ABCR1 mixture ratio of SDS + Ni(II) + Cd(II) mixture for *A. seifertii*, indicating additive effect of the mixture components.Although SDS and metal ions may have different modes of action against the bacterium, significant difference did not

however exist between predicted values of mixture toxicity on the bases of CA and IA in ABCR3 mixture ratio of SDS + Co(II) + CdII) mixture against*A. seifertii*.Similar insignificant differences between mixture toxicity predicted on the basis of CA and IA models for phenolic compounds with similar and dissimilar mechanisms of action was reported by Huang *et al*. (2011).

In addition, both CA and IA models predicted similar toxicities for the ternary mixtures of SDS + Ni(II) + Cd(II) and SDS + Co(II) + Cd(II). Identical toxicities predicted from CA and IA models for mixtures of similar-acting phenylurea derivatives were reported (Backhaus *et al*., 2004). This shows that both concepts, concentration addition and independent action, may serve as veritable tools for predicting toxicity of chemical mixtures (Faust *et al*., 2000). Also, the values of EC_{50S} predicted for SDS + Ni(II) + Cd(II), SDS + Co(II) + Pb(II) and SDS + Co(II) + Cd(II) ternary mixtures from CA model are not too far from those predicted from IA model. The ratio of CA-EC_{50} to IA-EC_{50}against*A. seifertii*varied from 0.877 ± 0.047 to 0.915 ± 0.021, 0.728 ± 0.361 to 0.813 ± 0.177 and 0.844 ± 0.088 to 0.906 ± 0.026, with average of 0.889 ± 0.018, 0.785 ± 0.041 and 0.887 ± 0.029, respectively for those ternary mixtures. Similarly, the ratio for *S. marcescens* (SerEW01) varied from0.722 ± 0.219 to 0.729 ± 0.164, 0.593 ± 0.420 to 0.632 ± 0.395 and 0.625 ± 0.334 to 0.642± 0.341 with average of 0.725 ±0.003, 0.614 ± 0.017 and 0.634 ± 0.007 respectively for the same ternary mixtures. These indicate that CA and IA models may have similar capability in predicting the toxicity of SDS and metal mixturesagainst*A. seifertii*but not *S. marcescens* (SerEW01).

In quaternary mixtures of three heavy metals and SDS, there was consisitency in the trend of sensitivity and tolerance between the planktonic and sediment bacteria. Similarly, the toxicities of the quaternary mixtures were higher than those of the ternary mixtures of the

toxicants.Furthermore, both he planktonic and sediment bacteria were more sensitive to the quaternary mixtures of SDS and metal ions than the ternary mixtures.This is reflected in their observed toxicity thresholds (EC_{50S}). Generally, the toxicity of the quaternary mixtures on both organisms also seems to increase as the proportions of the most toxic components (heavy metals) increase, with the corresponding decrease in the proportion of the SDS.This shows that the modulating effects of SDS on the mixture components tend to decrease with increasing complexity of the mixture and could also vary with mixture components and the proportions of those components in the mixture.

Although toxic index (TI) and modeldeviation ratio (MDR) analysesindicated synergism for both *S. marcescens* (SerEW01) and *A. seifertii*, the planktonic bacterium showed strongersynergistic interaction.Similar trend was also observed in ternary mixtures of the toxicants. This could be attributed to the reported greater sensitivity of planktonic organisms to aquatic toxicants in comparism to sediment dwelling organisms (Nweke *et al*., 2007a).However,additive effect was robserved in the ternary mixture of SDS+Ni(II)+Cd(II) against*A. seifertii*, while all quaternary mixtures of the toxicants showed synergistic effect against the same organism. According to Chen*et al.*,(2015), the complexity of any mixture tends to increase the relevance of synergistic effects. The observation in the present study agrees with this assertion. Synergistic interaction has been reported for quaternary mixtures of carbofuran, fenamiphos, formetanate and propamocarb, in a study on the toxicity of pesticides in wastewater by Fernández-Alba*et al.*,(2001). Similarly, synergism was also observed in the quaternary combination of antifouling biocides on the brine shrimp *Artemiasalina* (Koutsaftis and Aoyama, 2007).In addition, Hagopain-Schlekat *et al*. (2001) in a study on acute toxicity of five sediment-associated metals to *Amphiascus tenuiremis,*reported eqiutoxic mixture of

Pb+Cu+Zn+Ni to be synergistic.However, both synergistic and additive interactions have been reported for various studies on quaternary mixtures of heavy metals by different authors (Xu *et al.*,2011; Lin *et al*., 2016; Nweke *et al*., 2018).

In all quaternary mixtures, the CA and IA models greatly underestimated the joint toxicities of SDS and metal ions to *S.marcescens* (SerEW01), even at low concentrations. Underestimation by both models has been reported against *Vibrio qinghaiensis*, in a study that predicted the synergistic toxicity of heavy metals and ionic liquids on photobacterium Q67 (Ge *et al.,* 2014). However, for *A. seifertii*, in SDS + Cd(II) + Zn(II) + Pb(II) quaternary mixture, the CA model almost correctly predicted the experimentally-derived data at low concentration while slightly predicting higher toxicity at higher concentration. Similarly, in SDS + Cd(II) + Co(II) + Pb(II) quaternarymixture, both models slightly predicted higher toxicities at low concentration, while underestimating the joint toxicites at high concentrationin all but EECR50 mixture ratio. Gikas (2007), reported synergistic toxicity of binary mixtures of Ni(II) and Co(II) against growth of activated sludge microbial community. In the same study, Ni(II) and Co(II) mixture was antagonistic at the zone of decreasing stimulation. This observation indicated that the overall effect of toxicant mixture may vary with the threshold under consideration. Similar observation was reported by Nweke *et al*. (2018).In addition, in SDS + Cd(II) + Co(II) + Pb(II) and SDS + Cd(II) + Ni(II) + Pb(II) mixtures, both CA and IA predicted identical toxicities, against *A. seifertii,* as their dose-response curves were almost superimposed.

In the quinary mixtures in the present study, the consisitency in sensitivity of the planktonic bacterium against the sediment bacterium was still observed. Similarly, the toxicities of the quinary mixtures against both bacteria were higher than those of the preceeding quaternary mixtures of the toxicants. This is also reflected in their observed toxicity thresholds (EC_{50S}).

The toxicity thresholds (EC_{50S})of most of the quinary mixtures of the toxicants against*S. marcescens* (SerEW01)showed more consistent pattern with the corresponding increases in the proportions of the metal ion components and decreases in the proportions of SDS. The same however cannot be said of *A. seifertii.* Inconsistency in the pattern of mixture toxicities with respect to the amount of most toxic component present, have been reported (Fernández-Alba, 2001). However, Ribo and Rogers (1990), reported mixture toxicity correlation with the weighted sum of toxicities of individual components present.

The toxic index and model deviation ratios in all quinary mixtures showed strong synergistic interactions for the joint mixtures of the toxicants against both bacteria. This observation lends credence to the assertion that the relevance of synergistic effects increases with the complexity of the mixture (Chen *et al*., 2015). Rodea-Palomares *et al*. (2010),investigated a complex mixture including pharmaceuticals anda real wastewater sample on thecyanobacterium *Anabaena* CPB4337and concluded that synergismwas the predominant interaction in a wide range of the effect levels.Similar strong synergistic interaction was reported in a study on the combined toxicity of Pb+Cd+Hg+Ni+Cr mixtures against liver cells by Lin *et al*. (2016).However, Otitoloju (2003),in a study on thefixed-ratio of Pb+Cd+Hg+Cu+Zn mixture, according to their proportionsin Lagos lagoon sediment, reported antagonistic effect against benthic animals.Although these mixtures had somesimilar components with the toxicants in the present study, they are not exactly the same.These differencesin toxicant components and the test organisms could account for the differences observed in the studies.

In all the studied quinary mixtures, the CA and IA model grossly underestimated the joint toxicities of SDS and metal ions mixturesagainst*S. marcescens* (SerEW01), while slightly underestimating the mixtures' toxicities against*A.seifertii***.** Such underestimation of toxic effects

by combined effects of multicompound mixtures on the marine algae *Skeletonema pseudocostatum* by CA has been reported (Petersen*et al.*,2014).In addition, ABCR2 mixture ratio of SDS + Cd(II) + Zn(II) + Pb(II) + Co(II) quinary mixture showed biphasic effects upon exposure to *A. seifertii*. Biphasic response to chemicals is a phenomenon widely reported in microorganisms and higher forms of life (Calabrese and Blain 2005). Stimulation of dehydrogenase activity at low concentrations (hormesis) and inhibition at high concentrations has been reported for some of the mixture components on microorganisms (Tozum-Calgan and Atay-Guneyman, 1994; Gikas, 2007; Hashida and Inouye, 2007; Nweke *et al*., 2007a;). However, stimulation of the dehydrogenase activity in quinary mixturesof these toxicants at low concentrations against*A. seifertii* is not quite understood.

In the present study, the consisitency in the higher tolerance of the sediment bacterium against the planktonic bacterium to the senary mixtures of the toxicants was still observed. Similarly, the toxicities of the senary mixtures against both bacteria were higher than those of the quinary mixtures of the toxicants, as reflected in their experimentally-observed toxicity thresholds (EC_{50S}). This observation is however contrary to the report that the toxicity of mixtures decreases with increase in the complxety of the mixture. The toxic index and model deviation ratios in the senary mixtures showed strong synergistic interactions for the joint mixtures of the toxicants, for both organisms, exceptin ABCR3 mixture ratio against*A. seifertii* that showed marginal synergistic interaction.In a study on the synergistic toxicity of the multiple chemical mixtures, Chen *et al*., (2015), reported all the six-compoent mixtures of the toxicants to be strongly synergistic against earthworm.This result lends credence to the assertion that the relevance of synergistic effects increases with the complexity of the mixture (Chen *et al*., 2015).However, Verslycke *et al*. (2003), reported acute 96-hours toxicity of the equitoxic

mixture of Pb+Cd+Hg+Cu+Zn+Ni to be antagonistic against estuarine mysid. It is importany to note that this mixture had similar but not exactly the same components as the mixture in the present study. Thus these variations in the toxicant components and the test organims coud explain the different effect observed compare to the present study.

In all SDS + Cd(II) + Zn(II) + Pb(II) + Co(II) + Ni(II) senary mixtures, the CA and IA model grossly underestimated the joint toxicities of SDS and metalions mixtures to*S. marcescens* (SerEW01).However, against*A. seifertii*, in ABCR2 and ABCR3 mixture ratio, both models slightly overestimated the toxicity at low concentrations while underestimating the joint toxicities at high concentrations. Nweke *et al*. (2018), reported that both CA and IA models underestimated toxicity of a specific mixture ratio at low doses and overestimated toxicity at high doses, in their study on the toxicity of four metals and their mixtures to *Pseudomonasfluorescens*.These observations indicate that the overall effect of toxicants mixtures may vary with the threshold under consideration.Similar underestimation of toxic effects on the combined effects of pharmaceuticals, personal care products, biocides and organic contaminant multicompound mixtures on the marine algae *Skeletonema pseudocostatum* by CA has been reported (Petersen *et al*. 2014).

5.2. CONTRIBUTIONS TO KNOWLEDGE

This study is a base line survey on the anionic surfactants contents of Otamiri river water and sediment. It highlighted the fact that co-contamination of aquatic environment by heavy metals and SDS could be detrimental to the bacterial flora of the aquatic ecosystems. Similarly, the work has shown that SDS modulate the toxicity of heavy metals in aquatic environment. In

addition, the study showed that some heavy metals and SDS mixtures can be both stimulatory and toxic, depending on the mixture type and concentration involved. It has equally lent credence to the report by other researchers on the need for mixtures studies intoxicity testing, rather than focusing on individual toxicants, especially in aquatic environment. Furthermore, the planktonic bacterium (*Serratia marcescens* (SerEW01)) was more sensitive to the effects of these aquatic pollutants than the sediment bacterium (*Acinetobacterseifertii*).

5.3. CONCLUSION

Iron, mercury, cadmium, lead, nickel, zinc, cobalt and copper were the heavy metals identified in Otamiri river water and its sediment. Similarly,Pb, Cd, Ni, Hg, conductivity and turbidity recorded values higher than WHO recommended quality standards for drinking water, in the river water.Sodium dodecyl sulfate (SDS) was the predominant anionic surfactant present in both the river and sediment.*Serratia marcescens* (SerEW01) and *Acinetobacterseifertii* were the preponderant bacteria in Otamiri river water and sediment respectively. The toxicity assay showed that among the individual toxicants, the order of decreasing toxicities was Zn(II) > Cd(II) > Co(II) > Ni(II) > Pb(II) > SDS against*S. marcescens* (SerEW01) and Cd(II) > Co(II) > Zn(II) > Pb(II) > Ni(II) > SDS against*A.seifertii.*In addition,in all the indidvidual and various mixtures of the SDS and heavy metals tested, *S. marcescens* (SerEW01) were more sensitive to the toxicants and their mixtures than *A. seifertii*.Futhermore, though hormesis wereencountered in few mixtures at low concentrations; most mixtures however inhibited dehydrogenses activities in both bacteria at low and high concentrations. Similarly, both concentration addition and independent action models underestimated the toxicities of the SDS + heavy metal mixtures to both bacteria in most mixtures. The interactive effects of SDS + heavy metals mixtures to the two bacteria were mostly synergistic, thus suggesting possible detrimental

effects of co-contamination of Otamiri river ecosystems by SDS and heavy metals on the bacterial biodiversity of the river.

5.4. RECOMMENDATIONS

In view of the findings in this research, the following recommendations are made to mitigate and avert the environmental hazards associated with the uncontrolled dumping of untreated wastes and heavy metals into Otamiri river.

1. The dumping and subsequent burning of solid wastes at Otamiri river banks should be stopped.
2. All sand mining activities in the river should be stopped.
3. Periodic checks should be conducted to accertain the levels of heavy metals and anionic surfactants in Otamiri river water and sediment, as well as groundwater sources in Owerri and its environs.
4. Government should enforce the total relocation of Nekede auto-mechanics and artisans' workshops to the new site at Avuh, Owerri West L.G.A.
5. As a long term plan, government should consider building waste treatment facilities to reduce the indiscriminat discharge of untreated sewage into the river.
6. Further studies should be conducted on the toxicities of SDS and heavy metal mixtures to microbial community, algae and higher organisms from the river and sediments.
7. Periodic checks should be conducted to accertain the heavy metals and anionic surfactants contents in vegetables and crops cultivated along the banks of the river and irrigated with the river water.

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APPENDIX I

Plate i. Sand Mining/Dredging in Otamiri River, adjacent Mechanic Village, Nekede

APPENDIX II

Plate ii. Solid Wastes Dump at Otamiri River Bank (Free Zone, Mechanic Village, Nekede)

APPENDIX III

16S rRNA partial gene sequencing report for *Acinetobacter seifertii*

Standard ID

16S rRNA Full Sequencing — Humanizing Genomics macrogen

16S rRNA service report

Order Number : 180512FN-046
Sample name : A_contig_1

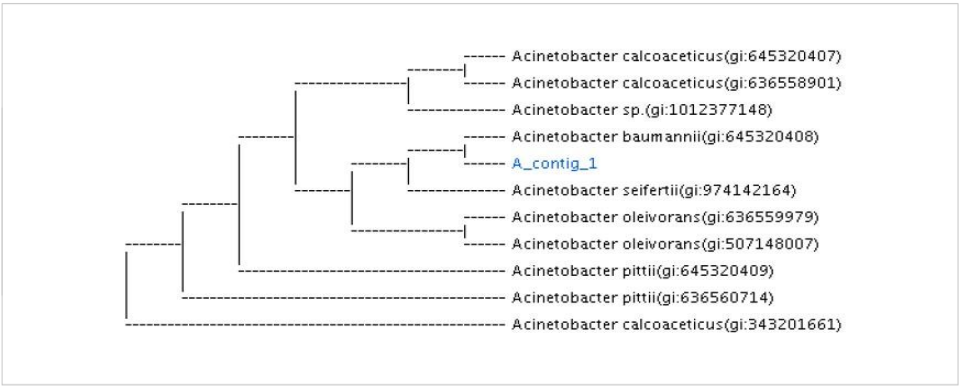
Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_134684.1	Acinetobacter seifertii	1460	1	1460	100	2641	0.0	1450/1460	99

Kingdom	Family	Genus	Species
Bacteria	Moraxellaceae	Acinetobacter	Acinetobacter seifertii



Characterization

Acinetobacter sp. play a significant role in the colonization and infection of patients admitted to hospitals. Their predominant role is as agents of nosocomial pneumonia

under investigation

APPENDIX IV

Bacterial Isolates from Otamiri River Water and Sediment and their Percentage Occurrences

Sample/Bacteria	% Occurrence
River water	
Staphylococcus	22
Enterobacter	11.11
Serratia marcescens (SerEW01)	33.33
Streptococcus	22.22
Escherichia coli	11.11
Sediment	
Streptococcus	5.30
Pseudomonas	10.53
Klebsiella	10.53
Acinetobacter seifertii	42.10
Bacillus	15.80
Escherichia coli	15.80

APPENDIXV

MTT Trial Runs Showing Toxicants Concentration Ranges

Toxicant						Conc. Range (mM)
Concs (mM)	0	0.1	0.2	0.5	1.0	
Zn	++++	+++	++	+	s+	Extended 0 - 1.5
Ni	++++	s+	s+	-	-	Extended 0 - 1.0
Cd	++++	+++	++	-	-	Extended 0- 01
Co	++++	s+	-	-	-	Extended 0-0.2
Pb	++++	+++	++	-	-	Extended 0 -1.0
Conc (mM)	0	1	2	3	4	
SDS	++++	+	++	++	-	Extended 0-10

KEY: + = colour intensity, s+ (small plus) = colour intensity was much reduced

Dilution formular:

$C_1V_1 = C_2V_2$

Where

C_1= concentration of the stock

V_1= volume of the stock to be used for further dilution

C_2 = concentration to be used for the toxicity assay

V_2 = total volume of the toxicant to be dispensed in the toxicity assay

APPENDIXVI

Preparation of Stock Solutions of the Toxicants (Heavy Metals, SDS and MTT-Indicator)

Metals	Molecular Weight (g/mol)	Stock Concentrations (mM)
$NiSO_4.6H_2O$ as Ni(II)	262.86	10
$CdSO_4.8H_2O$ as Cd(II)	256.50	10
$Zn(NO_3)_2.6H_2O$ as Zn(II)	297.49	10
$Pb(NO_3)_2$ as Pb(II)	331.21	10
$CoCl_2$ as Co(II)	129.84	10
SDS	288.38	50

To Prepare 10 mM Nickel Stock

262.86g/L = 1000 mM (1Molar)

2.6286g/L = 10 mM

1000 ml = 2.6286g = 10 mM

100 ml = 0.263g/100ml = 10 mM

To Prepare 10 mM Cadnium Stock

256.50g/L = 1000 mM (1Molar)

2.5650g/L = 10 mM

1000 ml = 2.5650g = 10 mM

100 ml = 0.257g/100ml = 10 mM

To Prepare 10 mM Zinc Stock

297.49g/L = 1000 mM (1Molar)

2.975g/L = 10 mM

1000 ml = 2.975g = 10 mM

100 ml = 0.298g/100ml = 10 mM

To Prepare 10 mM Lead Stock

331.21g/L = 1000 mM (1Molar)

3.3121g/L = 10 mM

1000 ml = 3.3121g = 10 mM

100 ml = 0.331g/100ml = 10 mM

To Prepare 10 mM Cobalt Stock

129.84g/L = 1000 mM (1Molar)

1.2984g/L = 10 mM

1000 ml = 1.2984g = 10 mM

100 ml = 0.130g/100ml = 10 mM

To Prepare 50 mM SDS Stock

288.58g/L = 1000 mM (1Molar)

14.419g/L = 50 mM

1000 ml = 14.419g = 50 mM

100 ml = 1.442g/100ml = 50 mM

Preparation of 0.1% MTT-Indicator Stock

This was done by dissolving 0.1g of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide granules in 20 ml of sterile deionizes water and made-up to 99.9 ml with sterile deionizes water

APPENDIXVIIa

Protocol for the preparation of Varying Concentrations of Pb(II)

Stock					1 mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.5	0.6
Volume of Water (ul)	1300	1100	1000	900	800	700	600	500	300	100
Volume of Toxicant (ul)	0	200	300	400	500	600	700	800	1000	1200
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIb

Protocol for the Preparation of Varying Concentrations of Cd

Stock		0.1mM					1mM			
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	0.002	0.004	0.008	0.01	0.02	0.04	0.08	0.1	0.2
Volume of Water (ul)	1300	1260	1220	1140	1100	900	1220	1140	1100	900
Volume of Toxicant (ul)	0	40	80	160	200	400	80	160	200	400
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIc

Protocol for Preparation of Varying Concentrations of Co(II)

Stock					1mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	0.05	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.6
Volume of Water (ul)	1300	1200	1140	1100	1000	900	700	500	300	100
Volume of Toxicant (ul)	0	100	160	200	300	400	600	800	1000	1200
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIId

Protocol for Preparation of Varying Concentrations of Ni(II)

Stock	1mM	10 mM							
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.8	1
Volume of Water (ul)	1300	1140	1100	1000	900	1270	1260	1240	1220	1200
Volume of Toxicant (ul)	0	160	200	300	400	30	40	60	80	100
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIe

Protocol for Preparation of Varying Concentrations of Zn(II)

Stock		0.1mM					1mM			
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	0.02	0.04	0.08	0.1	0.2	0.5	1	1.2	1.5
Volume of Water (ul)	1300	1260	1220	1140	1100	1260	1200	1100	1060	1000
Volume of Toxicant (ul)	0	40	80	160	200	40	100	200	240	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIf

Protocol for Preparation of Varying Concentrations of SDS

Stock				50mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0	1	2	3	4	5	6	8	9	10
Volume of Water (ul)	1300	1260	1220	1180	1140	1100	1060	980	940	900
Volume of Toxicant (ul)	0	40	80	120	160	200	240	320	360	400
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIg

Protocol for Preparation of Varying Concentrations of SDS+Ni and SDS+Cd(II) Binary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	120	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIh

Protocol for Preparation of Varying Concentrations of SDS+Ni(II) and SDS+Cd(II) Binary Mixtures (repeat)

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.1	0.2	0.4	0.6	0.8	1	1.5	2	2.5	3
Volume of Water (ul)	1280	1260	1220	1180	1140	1100	1000	900	800	700
Volume of Toxicant (ul)	20	40	80	120	160	200	300	400	500	600
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIi

Protocol for Preparation of Varying Concentrations of SDS+Zn(II) Binary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1200	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	100	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIj

Protocol for Preparation of Varying Concentrations of SDS+Zn(II) Binary Mixtures (Repeat I)

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.1	0.2	0.4	0.6	0.8	1	1.5	2	2.5	3
Volume of Water (ul)	1280	1260	1220	1180	1140	1100	1000	900	800	700
Volume of Toxicant (ul)	20	40	80	120	160	200	300	400	500	600
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIk

Protocol for Preparation of Varying Concentrations of SDS+Zn(II) Binary Mixtures (Repeat II)

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.5	1	1.5	2	2.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1200	1100	1000	900	800
Volume of Toxicant (ul)	10	16	20	40	60	100	200	300	400	500
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIl

Protocol for Preparation of Varying Concentrations of SDS+Pb(II) Binary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1200	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	100	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIm

Protocol for Preparation of Varying Concentrations of SDS+Pb(II) Binary Mixtures (Repeat)

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.1	0.2	0.3	0.4	0.5	0.8	1	1.5	2
Volume of Water (ul)	1290	1280	1260	1240	1220	1200	1140	1100	1000	900
Volume of Toxicant (ul)	10	20	40	60	80	100	160	200	300	400
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIn

Protocol for Preparation of Varying Concentrations of SDS+Co(II) Binary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.6	0.8
Volume of Water (ul)	1290	1284	1280	1270	1260	1240	1220	1200	1180	1140
Volume of Toxicant (ul)	10	16	20	30	40	60	80	100	120	160
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIo

Protocol for Preparation of Varying Concentrations of SDS+Co(II) Binary Mixtures (Repeat)

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.6	0.8	1
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1200	1180	1140	1100
Volume of Toxicant (ul)	10	16	20	40	60	80	100	120	160	200
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIp

Protocol for Preparation of Varying Concentrations of SDS+Pb(II)+Zn(II)Ternary Mixtures

Stock					1mM				10mM	
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.02	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.8	1
Volume of Water (ul)	1300	1200	1140	1100	900	1240	1220	1200	1140	1100
Volume of Toxicant (ul)	0	100	160	200	400	60	80	100	160	200
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIq

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Zn(II) Ternary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.4	0.6	0.8	1	1.5	2
Volume of Water (ul)	1290	1284	1280	1260	1200	1180	1140	1100	1000	900
Volume of Toxicant (ul)	10	16	20	40	80	120	160	200	300	400
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIr

Protocol for Preparation of Varying Concentrations of SDS+Pb(II)+Ni(II) Ternary Mixtures

Stock				10mM						
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	400	60	80	120	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1% MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIs

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Ni(II) Ternary Mixtures

Stock	10mM									
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	120	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIXVIIt

Protocol for Preparation of Varying Concentrations of SDS+Co(II)+Cd(II) Ternary Mixtures

Stock					10mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	120	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIu

Protocol for Preparation of Varying Concentrations of SDS+Co(II)+Pb(II) Ternary Mixtures

Stock			10mM							
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	120	160	200	3000
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIv

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Zn(II)+Pb(II) Quaternary Mixtures

Stock		10mM								
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.6	0.8	1	1.5
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1180	1140	1100	1000
Volume of Toxicant (ul)	10	16	20	40	60	80	120	160	200	300
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIw

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Ni(II)+Pb(II) Quaternary Mixtures

Stock			10 mM							
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.6	0.8	1
Volume of Water (ul)	1290	1284	1280	1260	1240	1220	1200	1180	1140	1100
Volume of Toxicant (ul)	10	16	20	40	60	80	110	120	60	200
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIx

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Co(II)+Pb(II) Quaternary Mixtures (Repeat)

Stock					5mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.025	0.05	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.8
Volume of Water (ul)	1290	1280	1268	1260	1240	1220	1180	1140	1100	980
Volume of Toxicant (ul)	10	20	32	40	60	80	120	160	200	320
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIy

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Zn(II)+Pb(II)+Co(II) Quinary Mixtures

Stock					5mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.025	0.05	0.08	0.1	0.2	0.3	0.4	0.5	0.6	0.8
Volume of Water (ul)	1290	1280	1268	1260	1220	1180	1140	1100	1060	980
Volume of Toxicant (ul)	10	20	32	40	80	120	160	200	240	320
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIzi

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II) Quinary Mixtures

Stock					5mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.025	0.05	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.8
Volume of Water (ul)	1290	1280	1268	1260	1240	1220	1180	1140	1100	1060
Volume of Toxicant (ul)	10	20	32	40	60	80	120	160	200	240
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

APPENDIX VIIzii

Protocol for Preparation of Varying Concentrations of SDS+Cd(II)+Ni(II)+Pb(II)+Zn(II)+Co(II)Senary Mixtures

Stock					5mM					
Tube number	1	2	3	4	5	6	7	8	9	10
Concentration (mM)	0.025	0.05	0.08	0.1	0.15	0.2	0.3	0.4	0.5	0.8
Volume of Water (ul)	1290	1280	1268	1260	1240	1220	1180	1140	1100	1060
Volume of Toxicant (ul)	10	20	32	40	60	80	120	160	200	240
Volume of Medium (ul)	500	500	500	500	500	500	500	500	500	500
0.1%MTT (ul)	100	100	100	100	100	100	100	100	100	100
Culture/Organism (ul)	100	100	100	100	100	100	100	100	100	100
Total Volume (ul)	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000