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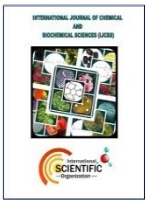

Quantitative Insights into Heavy Metal Bioremediation in Water and Soil Samples from Industrial Effluents and Computational Modeling of Microbial Toxin Proteins

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Abstract

Heavy Metals accumulation in water and soil has become a serious issue word wide. Heavy metals like Cu, Ni, Fe and Pb are known as potent toxins and environmental contaminant that also deteriorate human health. Moradabad or the brass city of India is also famous for the metal processing small units. During the course of metal processing a large number of heavy metals are released into the atmosphere and lithosphere. This place is also becoming a hot spot for E-Waste burning site and contributes to the increase in heavy metal concentration in air, water and soil. It has been strongly evidenced in many researches that microbial remediation can be a better alternate to reduce and removal of these heavy metals from the soil and water reservoir efficiently. In current research, homology modelling, identification and docking studies of natural inhibitors of the toxins produced by the identified bacteria is performed. Also, the quantitative analysis of heavy metals from the soil and water samples of 5 different industrial sites of Moradabad is performed. It was reported that the selected natural inhibitors can be utilized in real world to inhibit the toxins produced by the bacteria. The present study can be utilized for the heavy metal bioremediation on ground for the reclamation of soil and to improve the water quality of the natural reservoirs.

Keywords: Bioremediation, E-Waste Recycling, Environmental Contamination, Geogenic Sources, Groundwater Pollution, Heavy Metals, PCBs, Toxicity, Water Reservoirs

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1. Introduction

Heavy metals are metallic substances that have a comparatively higher density as compared to liquids. Heavy metals include metalloids like arsenic, which may produce toxicity at low exposure levels, according to the assumption that heavyweight and toxicity are connected. Associated with exposure has expanded significantly as a result of an explosive growth in their usage in a variety of manufacturing, Farming, Resident, and technical activities. Heavy metals have been recognized as coming from geogenic, domestic wastewater, and medicinal, agricultural, industrial, and atmospheric sources. Blast furnaces and foundries, mining, and other metal-based industrial operations all contributes considerably to pollutants. Moradabad, is known as brass city of India and is located in western part of Uttar Pradesh in India. It is well-known for its brass handicraft manufacture. Due to the higher cost of brass materials and un-employability among the metal industry workers, e-waste recycling becomes an alternate choice for the metal artisans. Moradabad receives a huge amount of e-waste, an approximate 50 percent of all waste PCBs, as well as 90 tons of e-waste burnt every day. Water reservoirs are final destination sink for all kind of pollutants. Contaminated water reservoirs degrade the quality of lithosphere. The heavy metals enter into the food chain and ultimately affects the human health. Water is a prevalent and very essential material on Earth for life. The dynamics of every living organism and ecosystem depends on water resources and their utilization. The majority of people use groundwater for household, agricultural, and industrial uses. Some settlements along the banks of the Ramganga and Gangan rivers use river water for agricultural purposes. The discharge of industrial effluent into rivers and lakes lacking sufficient treatments is the primary source of environmental contamination. Aside from the metals handicrafts production sectors, electronic waste recycling and metal waste recycling are performed out along the banks of the Ramganga River in Moradabad.

The trash is discharged onto the ground and into the sewers without being treated. Given the indiscriminate dumping of garbage, environmental protection in this area becomes critical. Moradabad's soil and water contains heavy metals that are harmful to the environment and may cause severe illnesses, especially cancer. There had been no research or medical evaluations to associate rising respiratory disorders with e-waste operations in the city, but there had been a rise in respiratory disorders instances among persons living in regions where PCBs were dismantled and segregated. Current study is conducted to explore the causes and remedy to overcome the environmental burden on natural resources and evaluation of quantitative estimation of heavy metal concentration in soil and water samples and possible remedy to inhibit the toxins produced by the indigenous bacteria in affected areas of natural ecosystem.

2. Materials and methods

Punetha *et al.*, (2015) inspected the heavy metal concentrations in commonly grown vegetables in Moradabad. Authors identified Cr, Mn, Zn, and Fe, in several non-edible and edible vegetables. In this study authors employed the “Atomic Absorption Spectroscopy (AAS)” to regulate the concentration of aforementioned heavy metals. It was concluded in their study that the concentration of Cr and Zn was crossing the maximum threshold limits set for human consumption [1]. Aprajita Singh *et al.*, (2018) investigated the concentration of heavy metals in Moradabad soil. It is well known that Moradabad is famous for brassware, industrial waste, and e-waste. Authors found that Fe, Cu, Ni, Zn, Cr, Cd, and Pb were heavily contaminating the soils in Moradabad, they also found that these heavy metals were mainly originating from printed circuit board recycling and burning. They employed the ICP-AAS method for the analysis after digestion. They recommended that there is an urgent need for the remediation of the contaminants from the soil to prevent further ecological disaster [2]. Animesh and Megha Agarwal (2014) conducted a study to investigate the effect of heavy metals on the aquatic life of Ramganga river in Moradabad. They conducted this study to analyze the effect of industrial waste, that is being discharged in the river, on aquatic life. They invested in three heavy metals i.e., Cr, Cu and Ni, and found that the Cr is the present in the maximum and lethal concentration. They also reported that the concentration of heavy metals decreases when they move from the river sides to the center. In the last they concluded that these industrial effluents have mixed solvents which increases the toxicity. Further these industrial effluents pollute the river severely and decrease the life in the river [3]. Moradabad is known for brass and e-waste, but these names are also associated with the heavy metals that heavily affect the living conditions in this area. Not only humans are affected but also the aquatic and edibles are also affected. Several research have been conducted to find the concentrations of heavy metals and found that concentration of heavy metals exceeds the normal limits several times. Though no study is conducted to find the solution to this problem. Current study is conducted to decrease the concentration of these heavy metals in soil and water. In current studies, bacteria are used to bio-remediate these heavy metals. These bacteria were identified from the soil and water samples of Moradabad. In-Silico studies were conducted to inhibit the toxins produced by these bacteria, so that these bacteria are not toxic for the environment. Natural inhibitors for the toxins produced by bacteria were identified. These natural inhibitors will inhibit the toxins and on the other hand bacteria will remediate the heavy metals from the soil and water making them suitable for crops and human health.

2.1. Methodology

2.1.1. Design

Contaminated soil and water samples are collected from various dumping sites from the areas located in various parts of Moradabad region. These all sites are all the small industrial sites situated in the Moradabad Region namely Mughalpura, Nawabpura, Peetal Nagari, Daulata Bagh, and Nagphani (Figure 1). Quantitative analysis was performed to identify the quantity of heavy metals in soil and water samples from sites. Also, the toxins of the identified indigenous bacteria were inhibited by the natural compounds to decrease the toxicity.

2.2.2. Sample and Instruments

Serial Dilution Technique, Gram Stain, Endospore Stain, Catalase Test, Glucose Test, and Mannitol Test were used for the identification of the bacteria from the different soil and water samples. Quantitative Analysis was also performed for the identification of toxic metals present in the soil and water samples of selected sites on the bank of river Ramganga.

2.2.2.1. Serial Dilution Technique

The serial dilution technique is employed to isolate microorganism colonies from soil samples.

2.2.2.2. Gram Stain

A Gram stain is a technique that looks for bacteria in areas where an infection is indicated, like the skin wounds, genitals, lungs, or throat.

2.2.2.3. Endospore Stain

Endospore staining is a distinct stain that identifies, recognizes, and differentiates an endospore from a vegetative cell.

2.2.2.4. Catalase Test

This test demonstrates the existence of catalase, an enzyme that catalyzes the oxygen release from hydrogen peroxide (H_2O_2).

2.2.2.5. Glucose Test

It evaluates an organism's potential to form glucose as well as transform pyruvic acid, the outcome of glycolysis, to gas by-products. It is a common tool for determining Gram-negative family Enterobacteriaceae, all of which are glucose fermenters although only some produce gases.

2.2.2.6. Mannitol Test

Mannitol is used to aid in the identification of an unidentified microorganism. Because coagulase, that disintegrates mannitol, is present in certain bacterial species, they may metabolize it via fermentation.

2.2. Data Collection

With the help of aforementioned tests several bacteria were identified in soil and water samples. A list of bacteria, toxins produced by these bacteria and what the naturally occurring inhibitors of these bacteria is given in Table 1.

2.3. Data Analysis

2.3.1. Quantitative Analysis

Table 2 shows the concentration of “Pb, Cd, Cu, Zn, Cr, Fe, Al, and Ni” found in water and soil samples. “Pb, Cd, Cu, Zn, Cr, Fe, Al, and Ni” were discovered in the following sequence in soil samples: “Ni < Cd < Cr < Al < Pb < Fe < Zn < Cu”. It was reported that the components of brass i.e., CuPbZn were in massive concentrations. Table 3 represents the concentration of the heavy metals (mg/kg) in water. The following sequence is obtained: “Ni < Cd < Cr < Al < Pb < Fe < Zn < Cu”.

2.3.2. Docked Structures

Figure 2 & Figure 3 represent the docked structure of the toxins produced by the bacteria and the identified natural compounds that inhibit these toxins. It was reported that there were 7 different bonds between Hemolysin and Carvacrol, 6 different bonds between Type 1 Toxin (txpa) and Cinnamic acid, 8 different bonds between α-Hemolysin and Resveratrol, 8 different bonds between Protective Antigen and Curcumin, 7 different bonds between Tetanus Neurotoxin and Berberine, and 6 different bonds between Vaginolysin and Carvacrol.

2.3.3. Amino Acid and Bond Type

With the help of visualizer software, the docked structures were analyzed and it was reported bonds are represented in the Table 4.

3. Results and Discussion

3.1. Hemolysin and Carvacrol

Bacillus cereus is a spore-forming food-borne bacterium that is often linked with milk, soup, vegetables, meat, rice and other dairy products. One emetic toxin (ETE) and 3 distinct enterotoxins are produced by B. cereus. Cytotoxin K, Nonhaemolytic enterotoxin (Nhe), and Hemolysin BL (Hbl), are three pore-forming enterotoxins that cause diarrhoeal food poisoning (CytK). It has been evidenced in several studies that Carvacrol, a naturally present chemical found mostly in the essential oils of thyme and oregano, is effective against the toxins produced by bacillus cereus [4,10-14]. In this study Carvacrol was investigated for its ability to reduce the toxin generated by Bacillus cereus.

3.2. Type 1 Toxin (txpa) and Cinnamic Acid

B. subtilis is considered a harmless bacterium because it does not have any disease-triggering features. It is not pathogenic nor toxic to people, animals, or plants. The potential danger of using these bacteria in fermentation facilities is minimal. Because of its propensity to create enterotoxins and emetic toxins, *Bacillus cereus* is the most common source of food poisoning in this category. Cinnamic acid is a low-toxicity aromatic compound that naturally exists. Humans have traditionally used it as a constituent of plant-derived fragrances and flavors. Cinnamic acid is a fragrance component that is often used in artistic cosmetic products, fine perfumes, conditioners, household cleaners, and certain other toiletries, as well as non-cosmetic items such as home cleansers and cleansers. Tonari *et al.*, (2002) found that certain cinnamic acids are effective against *Bacillus subtilis* and *Escherichia coli [5]*. Yilmaz *et al.*, (2018) also evidenced that certain cinnamic acids can be used as antibiotics to avoid the food poisoning caused by the *B. Subtilis* [15]. Many other studies have strongly evidenced that cinnamic acid is the natural inhibitor of *Bacillus subtilis*.

3.3. α-Hemolysin and Resveratrol

Staphylococcus aureus is a dangerous bacterium that causes a variety of severe ailments. A broad variety of virulence determinants, the most significant of which are produced toxins, determines S. aureus pathogenicity [16]. Toxins produced by S. aureus are categorized into 3 types: “superantigens (SAgs), exfoliative toxins (ETs), and pore-forming toxins (PFTs)”. Phenol-soluble modulins (PSMs) leukotoxins, Hemolysin-, and Hemolysin- (Hla or -toxin) are the four forms of pore-forming toxins [17]. Resveratrol is a phytoalexin that occurs naturally and belongs to the stilbene family of phenolic chemicals. Resveratrol has been found in over 100 medicinal and edible plants as a polyphenolic chemical, including Rheum rhamponticum, Cassia quinquangulata, Yucca shidigera, Arachis hypogaea, Polygonum cuspidatum, and many more [18-19] . Ma *et al.*, (2018) also reviewed that Resveratrol is effective against several foodborne pathogens like Campylobacter Sp., Staphylococcus aureus and E. coli [20].

3.4. Protective Antigen and Curcumin

Anthrax is an uncommon, possibly lethal illness caused by the Bacillus anthracis. as well as a possible bioterrorism threat Symptoms vary depending on the kind — cutaneous (skin), gastrointestinal, and inhalation [21]. It produces 2 toxins consisting of 3 proteins: “edema factor (EF), lethal factor (LF), and protective antigen (PA)”. The fatal toxin (LF+PA) causes submit mortality in animals, but the edoema toxin (EF+PA) causes edoema [22-23]. Curcumin occurs naturally in the form of curcuminoid chemical that was initially isolated from the plant Curcuma longa. The rhizome of this plant, in particular, is used to make turmeric, a spice that is a staple of several Asian diets. Curcumin has been used for centuries in traditional eastern remedies, even before contemporary identification of its chemical composition and functioning [24-25]. The active element in the eastern spice turmeric (Curcuma longa), curcumin (diferuloylmethane), has been demonstrated to block the activity of various enzymes and signaling molecules implicated in viral, bacterial and cancer infections, and inflammatory illnesses [26].

Antonelli *et al.*, (2014) evidenced that Curcumin is reducing the catalytic activity of the toxins produced by the B. anthracis [7].

3.5. Tetanus Neurotoxin and Berberine

Clostridium tetani is a common soil bacterium that causes tetanus. Tetanolysin and tetanospasmin are two exotoxins produced by C. tetani. Tetanospasmin is a neurotoxin that induces tetanus clinical symptoms [27-28].

Berberine is a chemical that has a bitter taste and a yellow hue. It may assist to strengthen the heartbeat, which may aid those suffering from certain cardiac diseases [29]. It may also help to destroy germs, manage how the organism consumes glucose from the bloodstream, and decrease swelling. Berberine is most typically used to treat diabetes, excessive cholesterol or even other fat concentrations in the blood, and elevated blood pressure. It is also employed to treat burns, canker sores, liver illness, and a variety of other diseases, however there is no evidence to back up a lot of these claims [30-33]. Skariyachan *et al.*, (2012) in a study utilized herbal compounds to inhibit Clostridium tetani [8]. They found that certain compounds have chemical composition like berberine that have the capability to inhibit Clostridium tetani.

3.6. Vaginolysin and Carvacrol

Gardnerella vaginalis is a bacterium that co - exist with several other bacteria in female vagina to maintain it infection-free. When too much gardnerella bacteria develops, you might get an illness called "Bacterial Vaginosis (BV)". BV is the most prevalent vaginal infection and may be readily treated with medications [34-37]. The pore-forming toxins, vaginolysin (VLY)and inerolysin (INY), generated by vaginal bacteria Gardnerella vaginalis and Lactobacillus iners [38-39]. It has been evidenced in several studies that Carvacrol, a naturally present chemical found mostly in the essential oils of thyme and oregano, is effective against the toxins produced by Gardnerella vaginalis [4,10-14].

3.7. Binding energy

For the Validation of the docked complex, the binding energy between the toxins and Natural compounds were calculated using the equation mentioned below:

$$\text{Binding Energy} = A + B + C - D$$

Where;

- A = Final Intermolecular Energy (vander Walls + H-bond + desolve + Electrostatic Energy).
- B = Final Total Internal Energy.
- C = Torsional Free Energy.
- D = Unbound System's Energy.

More binding energy depicts that the docked complex is more stable. As shown in Table 5, the binding energy between the Toxin and Natural Compound is sufficiently negative.

Table 1: Representing the Names of Bacteria and Natural Inhibitor of the Toxins produced by the Bacteria.

Bacteria	Toxins	Natural Inhibitor	References
Bacillus cereus	Hemolysin	Carvacrol	[4]
Bacillus subtilis	Type 1 Toxin (txpa)	Cinnamic acid	[5]
Staphylococcus aureus	α-Hemolysin	Resveratrol	[6]
Bacillus anthracis	Protective Antigen	Curcumin	[7]
Clostridium tetani	Tetanus Neurotoxin	Berberine	[8]
Gardnerella vaginalis	Vaginolysin	Carvacrol	[9]

Table 2: Representing the Concentration of the Heavy Metals (mg/kg) in Soil at Different Sites.

Site	Pb	Cd	Cu	Zn	Cr	Fe	Al	Ni
1	512	43	890	743	90	674	348	34
2	653	55	3092	2330	113	834	367	67
3	854	112	11203	7089	167	1334	753	94
4	587	103	2398	2050	194	2898	658	45
5	623	65	2100	2004	340	78	437	45

Table 3: Representing the Concentration of the Heavy Metals (mg/kg) in Water at Different Sites.

	Pb	Cd	Cu	Zn	Cr	Fe	Al	Ni
1	238	ND	378	265	23	23	132	ND
2	344	ND	650	322	57	93	238	ND
3	433	23	893	657	94	62	392	12
4	354	ND	349	589	81	102	341	ND
5	378	ND	388	324	40	17	429	13

Table 4: Representing the Number, Types and Amino Acids of the Formed Bonds between Toxins and Natural Inhibitors.

Bacteria	Toxin	Natural Inhibitor	Ligand : Amino Acid	Bond Type
Bacillus cereus	Hemolysin	Carvacrol	UNL1 : ASN37	3 H-Bond and 4 Hydrophobic Bond
			UNL1 : SER40	
			UNL1 : SER35	
			UNL1 : TRP80	
Bacillus subtilis	Type 1 Toxin (txpa)	Cinnamic acid	UNL2 : LEU34	4 H-Bond and 2 Hydrophobic Bond
			UNL2 : HIS40	
			UNL2 : LYS37	
			UNL2 : PHE32	
Staphylococcus aureus	α-Hemolysin	Resveratrol	UNL3 : TYR256	4 H-Bond, 1 Electrostatic and 3 Hydrophobic Bond
			UNL3 : GLN177	
			UNL3 : TYR191	
			UNL3 : ASN209	
			UNL3 : ARG200	
			UNL3 : THR199	
			UNL3 : LEU258	
Bacillus anthracis	Protective Antigen	Curcumin	UNL4 : THR624	4 H-Bond and 4 Hydrophobic Bond
			UNL4 : ILE689	
			UNL4 : TYR688	
			UNL4 : PRO692	
			UNL4 : LEU685	
Clostridium tetani	Tetanus Neurotoxin	Berberine	UNL5 : ALA69	2 H-Bond, 2 Electrostatic and 3 Hydrophobic Bond
			UNL5 : VAL171	
			UNL5 : LYS168	
			UNL5 : GLU271	
Gardnerella vaginalis	Vaginolysin	Carvacrol	UNL6 : TYR245	2 H-Bond and 4 Hydrophobic Bond
			UNL6 : ALA371	
			UNL6 : VAL176	
			UNL6 : LEU365	
			UNL6 : PRO370	
			:UNL1 - A:ALA371	

Table 5: Illustrating the Binding Energy of the Docked Complex between Toxins and Natural Compound.

Toxins	Natural Inhibitor	Binding Energy
Hemolysin	Carvacrol	-6.56
Type 1 Toxin (txpa)	Cinnamic acid	-4.97
α-Hemolysin	Resveratrol	-6.41
Protective Antigen	Curcumin	-5.05
Tetanus Neurotoxin	Berbine	-4.20
Vaginolysin	Carvacrol	-3.73

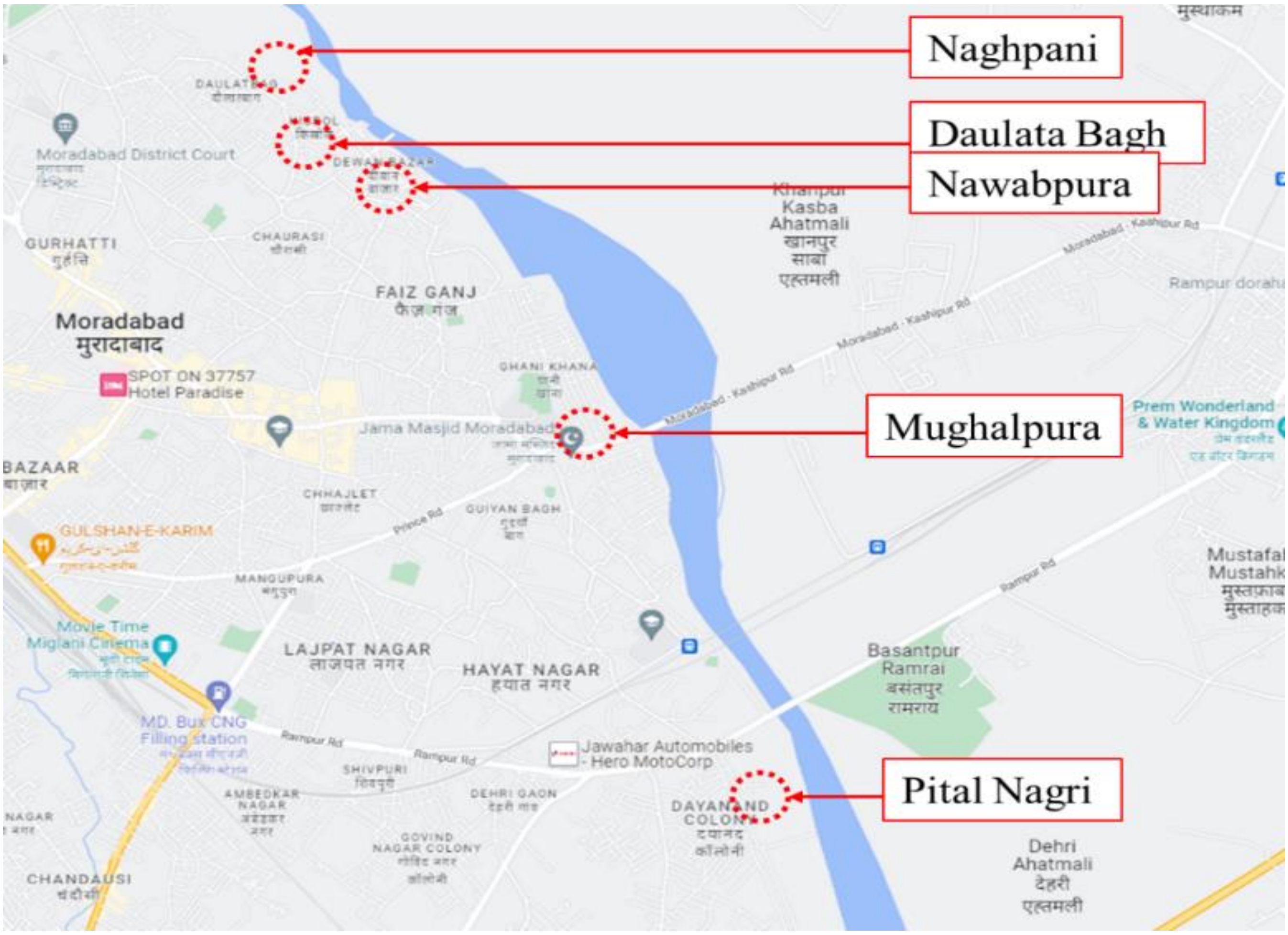

Figure 1: Representing the Different Sites in Moradabad near the Banks of Ramganga Selected for the Study.

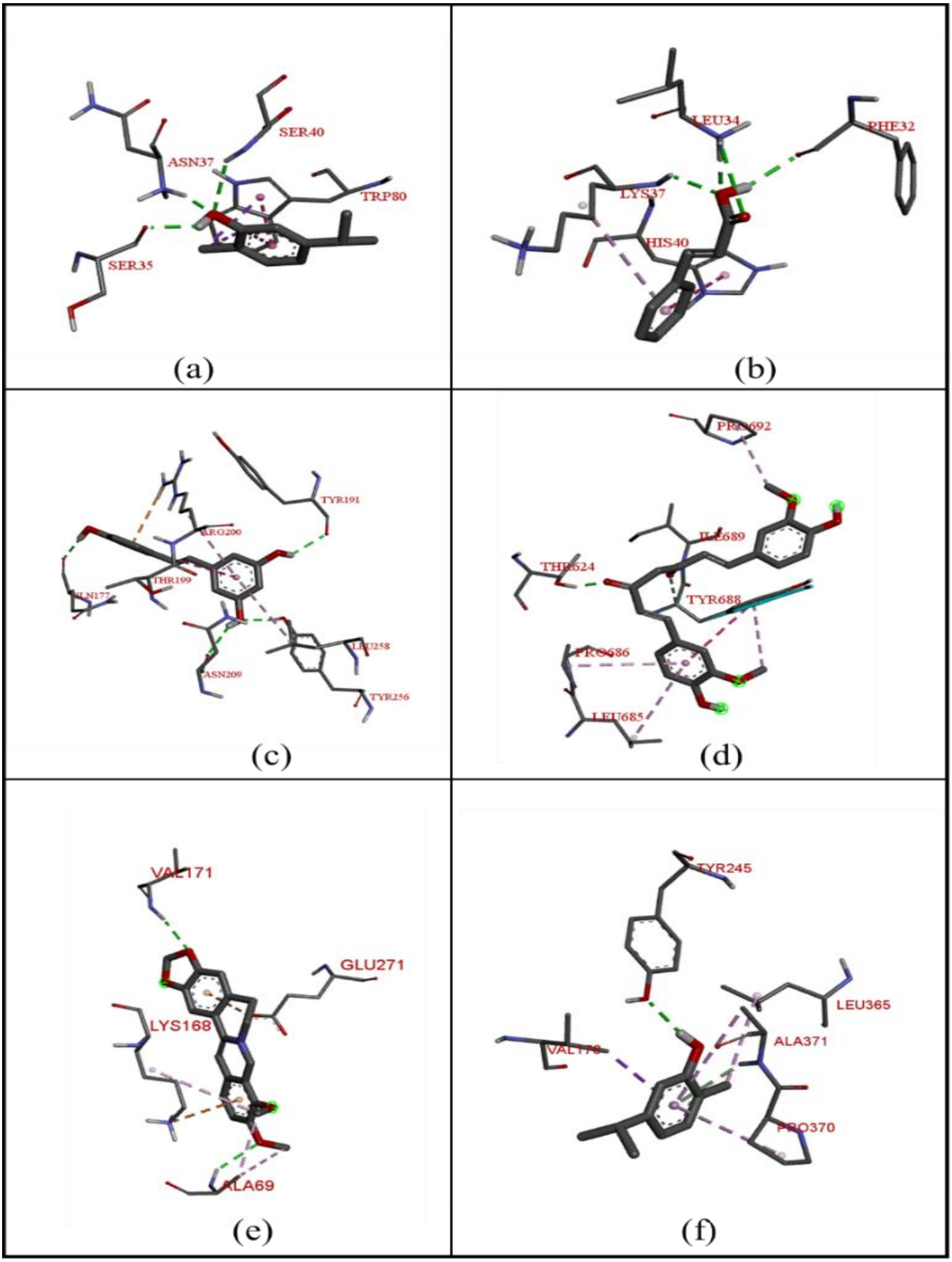

Figure 2: Representing the 3D Docked structure of Toxins Produced by Bacteria and their Natural Inhibitor: (a) Hemolysin – Carvacrol (b) Type 1 Toxin (txpa) – Cinnamic acid (c) α-Hemolysin – Resveratrol (d) Protective Antigen – Curcumin (e) Tetanus Neurotoxin – Berberine (f) Vaginolysin – Carvacrol

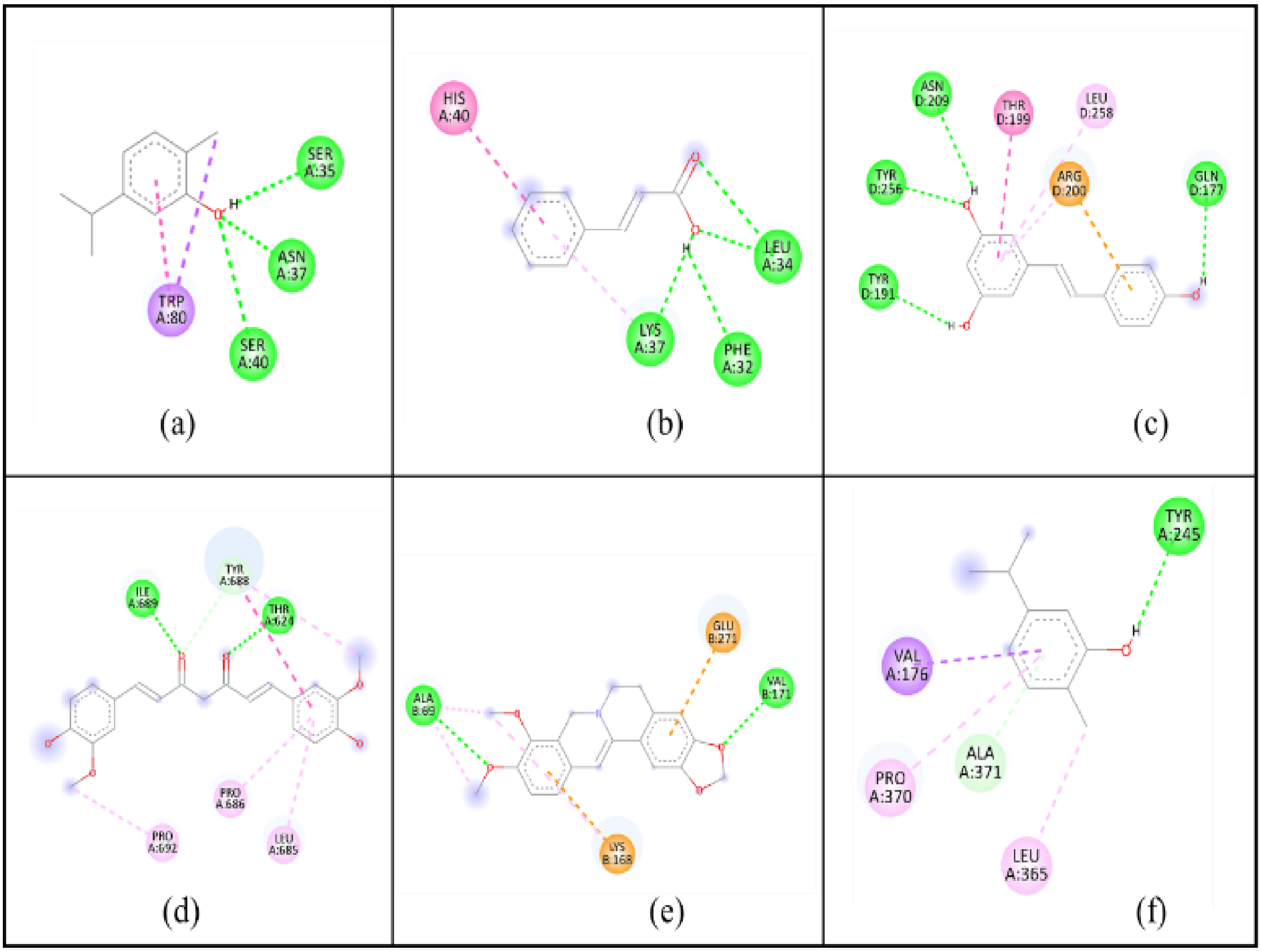

Figure 3: Representing the 2D Docked structure of Toxins Produced by Bacteria and their Natural Inhibitor: (a) Hemolysin – Carvacrol (b) Type 1 Toxin (txpa) – Cinnamic acid (c) α-Hemolysin – Resveratrol (d) Protective Antigen – Curcumin (e) Tetanus Neurotoxin – Berberine (f) Vaginolysin – Carvacrol.

4. Conclusion

According to studies, most e-waste recycling sites in Moradabad are unauthorized and they are using unmannered wrong practices causes health hazardous and contaminates the natural resources. Even underground water is also contaminated through leaching and percolation of heavy metal into water reservoirs. Because the impoverished do not have easy access to physicians, determining the health consequences of e-waste-related jobs proved challenging. Both recycling and dismantling are primitive processes. Consequently, 40- 50% of metals are left un-extracted, resulting in pollution. As per the study it has been established that several natural compounds can be used to increase the production of bacteria and inhibiting the toxins produced by them can be a promising approach to bio-remediate the heavy metals from the water and soil in such areas. According to several studies, it was also established that producers must be held accountable for pollution produced by their goods. Electronics producers must take accountability for their goods or e-waste will continue making its way to the informal sector of waste recycling.

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