Effect of Heavy Metal Contamination on Soil Enzymes Activities

Justice Ofori Yeboah, Guangyu Shi, Weilin Shi*

Department of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou, China
Email: oforiyeboahjust@gmail.com, shigylove@126.com, *shi@163.com

Abstract

Several enzymes catalyze much of the processes that exist in the soil. Enzymes in polluted soils are usually less active due to their exposure to heavy metals. The main goal of this study was to see how bioavailable types of Cd affected the behavior of catalase, urease, and dehydrogenases, as well as to compare the findings from naturally and artificially polluted samples. An experiment was conducted on two types of farmland (garden) soil: natural soil and soil that had been chemically polluted with Cd. The total content of heavy metal graded these soils as very highly polluted with Cd. The experiment was repeated four times to test the effects of increasing concentration and days (time). Extracellular enzymes from farmland performed enzymatic activity tests that lasted 6 to 29 days after soil sampling. After 0, 5, 10, 20, 30, and 45 days of incubation, soil samples were taken for testing respectively. However, even though no nutrient was added, dehydrogenase and urease activity increased as Cd concentration increased from 0 to 5 mg/L as the days passed. This is a result of enzymes engaging in respiratory and other living activities because of the low cadmium concentration and respiratory soil properties. However, there were significant variations in enzyme activity between naturally polluted and artificially contaminated soils. Dehydrogenases, Urease, and Catalase all showed a common pattern of enzyme sensitivity, which could be ordered as Dehydrogenase > Urease > Catalase. Dehydrogenase enzyme activity has been discovered to be more Cd resistant.

Keywords

Enzyme Activity, Dehydrogenases, Catalase, Urease, Soil Contamination, Heavy Metal

1. Introduction

A substantial percentage of the earth consists of soil that constitutes an essential

part and component of the ecosystem, which forms the material basis for human and plant existence. As human life, progresses with dramatically increased development and industrialization, heavy metals and soil contamination may continue to spread globally. Heavy metals are a specific group of elements with an atomic density of more than 6 g/cm^3. The global increase in human development is rapidly changing our world and has generated unique soils that vary in contaminant nature and concentration, moisture, and nutrient levels. The process of understanding how soil communities are affected by human impacts includes resolving differences in the structural and functional characterization of the biotic community. Within the soil, the presence and concentration of contaminants will modulate interactions between soil organisms (Fabietti et al., 2010).

Research on soil function, specifically enzymatic activity, in these systems, is an increasingly important approach to a more holistic understanding of soils in contaminated environments. In contaminated soils, the activity of the microbial community is intertwined with soil abiotic properties (Krčmar et al., 2018). Functions like cellulose degradation or nitrogen cycling can be measured by using soil enzymatic activities as indicators (Cofie et al., 2014). When microbial communities adjacent to industrialized sites were analyzed, they showed lower enzymatic function with higher soil metal loads as well as lower community diversity (Ahmad et al., 2018). Lower enzymatic activities have also been observed when the soil was experimentally contaminated with heavy metal loads. For instance, Kandeler et al. (1996) and (1999), showed lower urease, alkaline phosphatase, and xylanase activities for soils experimentally contaminated with Zn, Cu, Ni, V, and Cd compared to soils that were not experimentally contaminated.

However, in other studies, high soil metal loads were associated with high enzymatic activities and in other studies, shifts in the microbial community composition and functioning were found with a metal load. These results highlight the complexity of the effect of heavy metal contamination on soil enzyme activities and the need to consider the experimental details of each study. The goal of this research was to determine the relationship between elevated hazardous element (i.e., cadmium) concentrations in soil and extracellular enzyme activities that are proxy measures for nutrient cycling. The results of this study will help answer an important question relevant to restoration ecology: What are the effects of long-term metal contamination on microbial nutrient cycling in soils?

2. Material and Method

2.1. Site Description

The primary research site is located near the town of Suzhou (China's Jiangsu Province, Suzhou University of Science and Technology, (latitude N 34˚38', longitude E 119˚38'). The study area is a farmland situated in the warm-temperate, semi-humid, monsoon climate region (222 m·asl; T$_{winter}$ = 8˚C - 22˚C, T$_{summer}$ = 22˚C - 42˚C); several arboreal species dominated the area, including *Moringa oleifera*, Nimes plants, *Eugenia jambolana*, and the plant community is defined by

temperate, evergreen plants growing in diverse contamination (Cofie et al., 2014).

2.2. Soil Collection

Soil samples from the top 0 - 20 cm. The soil samples from the research region were packed into a light plastic container to the laboratory on ice, sieved with 2-mm mesh, and kept at a temperature of 20°C for enzyme assays of catalase, urease, and dehydrogenase activity. Extracellular enzyme activity was measured to determine the degree of enzyme activity as well as whether collected soil was already contaminated with other heavy metals. After the removal of the huge roots, the soil sample was used as control, after which further sampling was carried out with addition of contaminants and the contaminated samples were studied from days 6 to 29.

The soil properties were as follows: pH 6.16, CEC 17.04 CMoL·kg^{-1}, organic matter 4 0.05 g·kg^{-1}, N 0. 77 g·kg^{-1}, P 10.32 mg·kg^{-1}, and K 71.93 mg·kg^{-1}. rough particulates are first separated after natural air-drying and then screened through 100 mesh for standby. The soil sample was inspected over several days for effective data correctness, other analytical mistake was taken into consideration, as well as being separated into three independent samples to ensure no conflicting changes, with rises or declines in enzymatic activities during assays procedure. Cd with a background of 0.27 mg·kg^{-1}. Cd (NO$_3$)$_2$ was used to prepare the heavy metal salt solution.

Semi-micro Kjeldahl methodology was applied to determine nitrogen. The sulfuric acid-per-chloric acid digestion method was used to quantify total phosphoric acid, and organic matter determines by the $K_2Cr_2O_7$ volumetric method. The concentrations of Cd in dispelled soil samples was determined using the complete decomposition method and flame atomic absorption spectrophotometry, which never surpass the secondary norm of the "Environmental Quality Standard for Soils" (GB15618-1995) (Cd£0.3 mg/kg) or the first-grade standard of soil environmental quality in Jiangsu Province (Cd £0.2 mg/kg) (Shi & Ma, 2017). The concentration of other heavy metals was also determined by AA-7000 atomic absorption spectrophotometer (AAS, Shimadzu, Japan). The solution was mix into the air-dried soil and well mixed. After standing for 45 days, it was used as test soil.

2.3. Enzyme Activities and Basal Respiration Assays

As the research commenced other approach of testing enzymes was considered in order to accurately evaluate the efficacy of the enzymes' reactions. The indophenol-based colorimetric method was used to assess soil urease activity, whiles NH_4^+ was release by urease-mediated, the enzymatic hydrolysis of urea was measured calorimetrically at 578 nm (Guan, 1986; Guan et al., 1986). Dehydrogenase activity is determined by reducing 2,3,5-triphenyl tetrazolium chloride (TTC), extracting the triphenyl formazan (TPF), and measuring as 485 nm (Guan et al., 1986). Catalase activity was determined using the titration process (Guan et al., 1986), and soil basal respiration (SBR) is determined using the so-

dium hydroxide absorption method (CO_2 generation by incubation for 24 hours at 25˚C) (Xu et al., 2020).

2.4. Material and Reagent

The enzyme solutions to be used are Catalase, Urease, Dehydrogenase activity with different molarity, as the experiment proceeds all these soil enzymes were exposed to cadmium reaction.

2.4.1. Catalase Activity Determination

Volumetric method for measuring soil catalase

The catalase activity was determined using 1.5 mol/L H_2SO_4 solution, 0.3% percent H_2O_2, a saturated alum solution, and 0.02 mol/L potassium permanganate as reagents which was carried by out Zhou & Zhang (1980).

The experimental steps involve supplementing 2 g soil in Erlenmeyer flasks (50 mL) containing 40 mL and 5 ml 0.3% of distilled water and H_2O_2 solution respectively then sealed immediately. The Erlenmeyer flasks were put on the oscillator for (120 r/min). After 20 minutes, 1ml full-bodied aluminium potassium alum was added and the solution was immediately filtered in the triangle bottle containing 5 ml, and later 1.5 mol sulphuric acid was also added. Subsequently, the solution in the bottle is filtered with quantitative filter paper, 25 ml of the filtrate been absorbed, and titrated to purple red and 0.02 mol/L potassium permanganate added. Also, a soil-free control was performed.

2.4.2. Determination of Urease

Colorimetric method determination of soil urease

The solutions used for the Urease activity determination include Citrate solution with a pH of 6.7, along with Sodium phenol solution, Sodium hypochlorite solution, 10% urea solution is also used with a Standard solution of N as a reagent.

The Experimental steps involve supplementing 5 g soil in Erlenmeyer flasks (50 mL) containing 1ml toluene then gasser plug tightly and shake gently for 15 minutes. 10 mL, was added to 10% urea solution and 10 mL citrate buffer (pH 6.7) and mix carefully within the used bottle. Later put the Erlenmeyer flasks in a constant temperature box at 37 for 24 hours. The suspension is then filtered by diluting the scale with distilled water heated to 38 degrees Celsius (toluene should float above the scale). The suspension is shaken and filtered. The filtrate was then diluted with 20 mL of distilled water, later sodium phenol solution of 4 ml was added, and sodium hypochlorite solution of 3ml was included immediately. Upon adding each reagent, the mixture was shaken immediately. After 20 minutes, the mixture was diluted to the scale and the absorbance was recorded at 578 nm. Urease activity was calculated by subtracting the difference between the absorbance of the control sample and the amount of ammonia nitrogen according to the standard curve.

2.4.3. Dehydrogenase Activity Determination

In a tube with 1 g of soil sample cultured, 0.2 ml of 3% sterile triphenyl tetrazolium chloride (TTC) solution and 0.5 ml of 1% sterile glucose added to assess dehydrogenase activity. Following a 24-hour incubation time at 28˚C, 10 ml of methanol was added as reagents.

In this study, the dehydrogenase procedure includes: 50 ml beaker, 20 g of fresh soil was combined with 200 mg of dried $CaCO_2$ and brought to 90% water-holding capacity that contains 2.0 ml of a 1% TTC mixture. It was completely blended, with the surface tamped keeping the air out. After that, the sample was incubated with a pressurized incubator (R.H., 70) within 24 hours at 30˚C. During the period of incubation, the beaker was filled with a 25 ml volume of methanol and 5 minutes of stirring. The resulting slurry was filtered with successive aliquots of methanol through a Buchner filter (Whatman No. 5 paper). The amount of extractant used for each sample was then recorded.

Using methanol as a reference blank, the coloured density extract was measured spectrophotometrically at a wavelength of 485. Comparisons with a TPF standard curve in methanol were used to measure concentrations. The following equation was used to calculate the amount of hydrogen transmitted as TTC decreases to TPF in 20 g soil: 2,3,5-triphenyl tetrazolium chloride + 2H + triphenylformazan + HC1. 150.35 pH is needed to make 1 mg of TPF.

2.4.4. Soil Amendment and Incubation

The soil moisture adjusted to 60% water holding capacity with distilled water incubated at 25˚C was kept in darkness for 45 days with treatment repeated four times. A stock solution was developed after dissolving Cd 2.0 g/L in water. In a volumetric flask, dissolve 3.723 g Cd sulfate in 1000 mL water. Cd was applied to soil samples weighing 500 g at mass concentrations of 0 mg/kg (control sample), equally distributed of 0.0, 0.1, 1.0, 2, 5, 10 mg/L cadmium (II) concentrations of the Cd stock solution were sprayed. After soil amendment and incubation, 10 g grams of soil with six different levels was thoroughly mixed with 90 mL distilled water, respectively. 1 mL of the soil solution was then moved to a 100 mL conical flask and incubated at 28˚C for another 15 days. The sample was analyzed and the analytical error was also accounted for and divided into three separate samples. The experiment was carried out in 45 days with the activities and effects recorded.

Data processing; After that, using Microsoft Excel 2010, the data was analyzed and a line error analysis was performed (Figures 1-6). As the experiment went on, there were some errors and data reported. The outcome and data of the experimental processes of different concentrations of cadmium reactions to Catalase, Urease, and Dehydrogenase activity are shown in the diagram in the result and discussion.

3. Results and Discussion

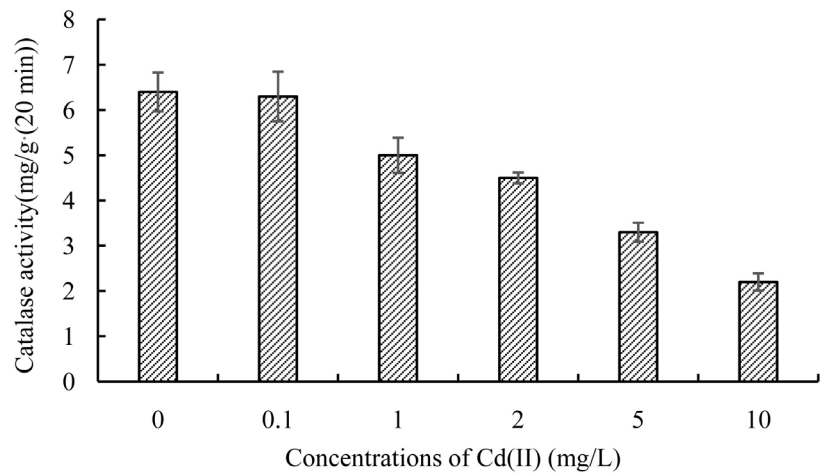The most significant metrics for evaluating and monitoring the effects of soil

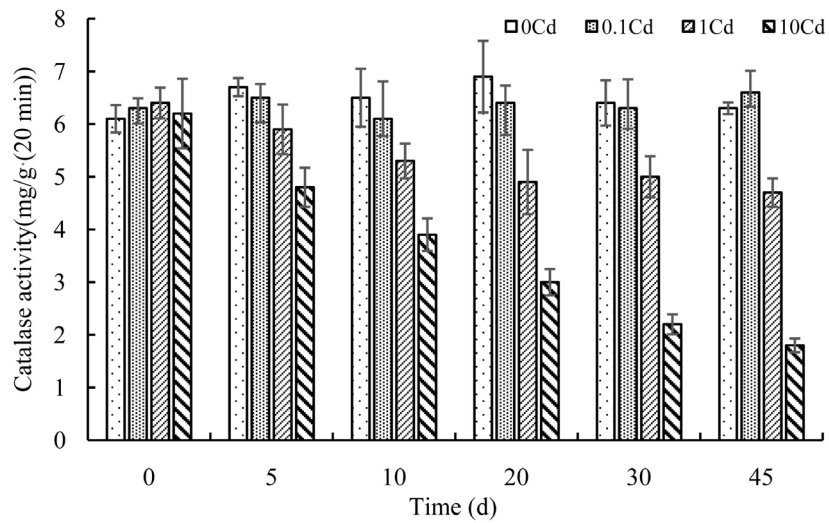**Figure 1.** Effect of Cd concentrations on Catalase activity in the soil.

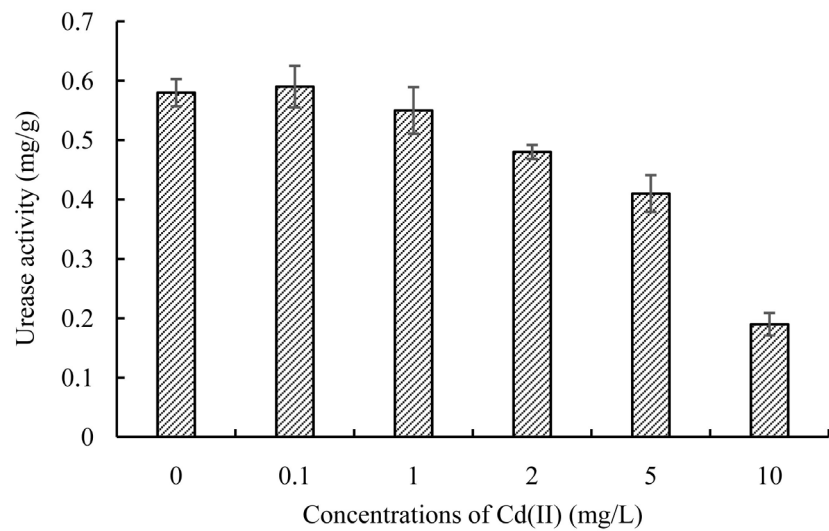**Figure 2.** Effect of Cd concentrations on Catalase activity with time (d).

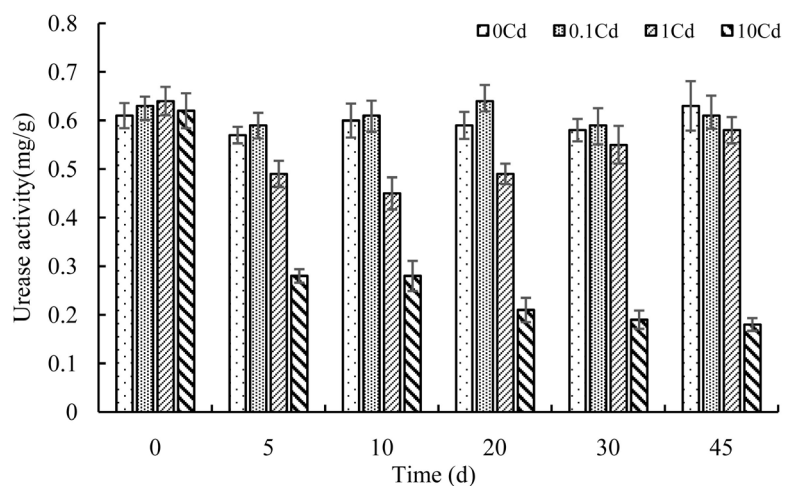**Figure 3.** Effect of Cd concentrations on Urease activity in the soil.

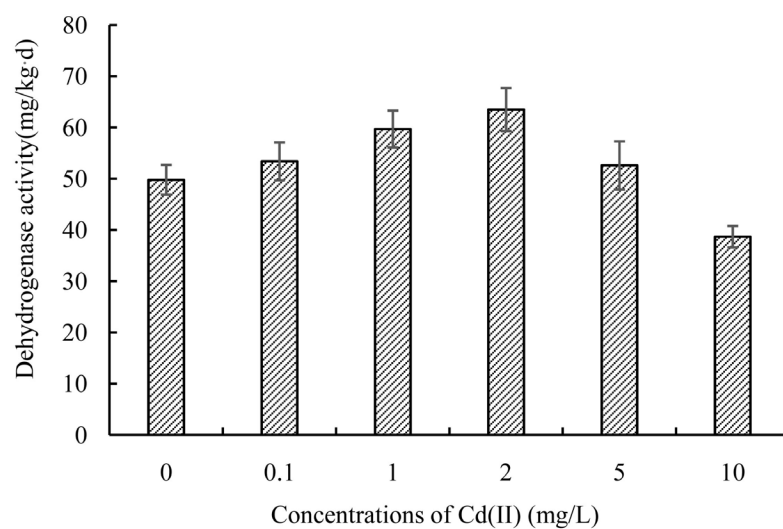**Figure 4.** Effect of Cd concentrations on Urease activity with time (d).

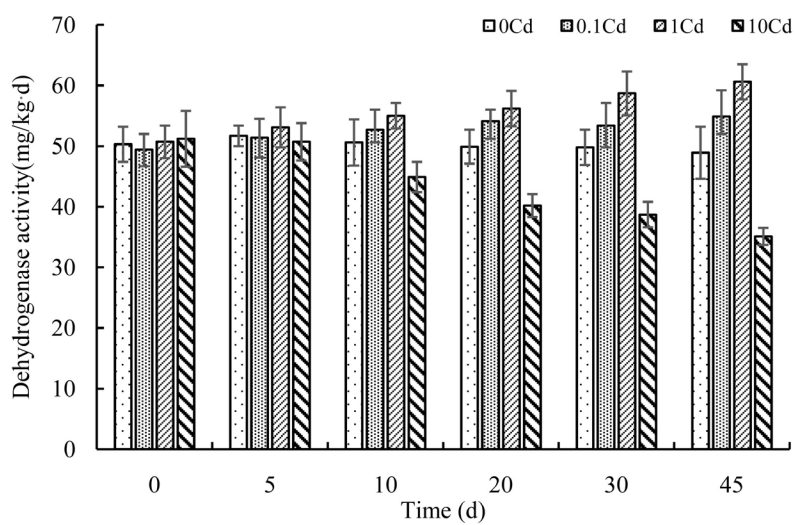**Figure 5.** Effect of Cd concentrations on dehydrogenase activity in the soil.

Figure 6. Effect of Cd concentrations on Dehydrogenase activity with time (d).

management, agricultural activities, or contaminants on soil health, according to (Yang et al., 2016) in his report, is enzyme activity as stated by (Oleszczuk et al., 2014). Indirectly, enzymatic activities represent and display the ability to self-purify the soil affect by pollution (Cui et al., 2013a). The toxicological effects of Cd on the structure and activity of soil microbial communities are shown to be highly dependent on Cd content and incubation duration, according to this research. The inhibition degree of three enzymes' activities, Catalase, Urease, and Dehydrogenase, varied substantially between different incubation durations.

Before the 45-days incubation period, microbial activities such as catalase, urease, and dehydrogenase activity showed rapid inhibition, indicating that the microbes could react to Cd as well as other metals when exposed. The rise of soil microbial activity inhibition is likely to be linked to the microbial community's sensitivity and adaptation, pollutant concentrations, and the mechanisms and concentration intensity of Cadmium as heavy metal.

Catalase, Urease, and Dehydrogenase activity were determined before the reaction, with each having a unique method of testing, and then combined with various concentrations of Cadmium in the treated soil samples, leading to a reduction in enzymatic activity as Cadmium concentration increases. Toxicity of Cd, Cu, and Pb known as trace heavy metals have a significant impact on soil enzymatic activity, which was measured using the dehydrogenase and urease enzymes (Chaperon & Sauvé, 2008).

It was discovered that metals resulted in reduction enzymatic activity and that the interaction of the metals occurs quickly enough to produce the observed results. According to the data collected, low cadmium concentrations did not affect enzyme activities, which would gradually decrease as cadmium concentrations rise, as will enzymatic activities. Influence on soil quality was found to affect soil enzymatic activities, soil respiration, and microbial abundance/biomass in previous studies (Bowles et al., 2014; Mbuthia et al., 2015). The most adverse influence of various contaminants related to soil health was estimated using soil enzymes such as soil urease activity, acid phosphatase activity, dehydrogenase activity, and catalase activity (Masto et al., 2009).

3.1. Effect of Cadmium on Catalase Enzyme Activity of the Experiment

Cadmium concentration with catalase activity

Catalase enzymes produce an anti-oxidant enzyme that breaks down hydrogen peroxide (H_2O_2) into water and oxygen while avoiding the formation of free radicals. Catalase breaks down peroxide, and its behavior is influenced by soil dehydrogenase, amidase, glucosidase, and esterase activity, as well as organic oxygen concentration, microbe biomass, and CO_2 shifts which enhances soil fertility (Burns et al., 2013). **Figure 1** shows the effects of Cd increasing concentration on catalase activity: enzyme activity was unaffected at concentration of 0.1 mg/L, however it quickly decreased at higher concentrations, showing a clear detrimental effect of cadmium on enzyme activity. Catalase activity was inhi-

bited in soils at the maximum Cd concentration levels. Excessive Cd concentrations were hazardous to microorganisms and had a higher impact on enzyme activities, with growing Cd concentrations in the soil reducing catalase activity.

Cadmium concentration and catalase activity are inversely and significantly related (r = X, df = Y, $P < 0.000X$), as previously observed by Yang et al. (2016). This demonstrates that Cd, has a strong impact on soil catalase, confirming its toxicity on soil microorganisms (Shi & Ma, 2017; Sun et al., 2016); causal relationship is attributable to microorganisms transferring the energy required for cell growth and maintenance to protection against toxicant effect of cadmium (Minnikova et al., 2017).

Heavy metals as contaminants interrelate with the enzyme-substrate complex, denature the enzyme, or interfere by protein active sites, resulting in decreased enzyme activity (Li et al., 2015). With an increase in Cd concentration and the loss of energy by Catalase enzymes due to the strain on the enzyme to sustain its cell, the process and activities in the soil gradually decrease. Catalase enzymatic activities decrease as the concentration rises with no additional nutrient or energy added to the soil.

Cadmium concentration to time and days on catalase activity

Several factors as Cd and soil type as well as climate influenced cadmium bioavailability (Dar, 1996; Dar & Mishra, 1994), and persistence in the ground is another major parameter; In Figure 5, we can observe a clear relation between the time Cd remains in the soil and enzyme response intensity.

Figure 2 below gives data on different concentrations of Cd with time and days as the experiment took place. The concentrations of Cd that changed significantly were recorded; the images below show Cd concentrations that changed significantly with catalase enzymes throughout 0 to 45 days.

The concentration of (0.0. 0.1, 1.0 10.0) g/mol in 0 to 45days had their respectively data readings on the catalase activity. For the same Cd concentration on day 10 of culturing, catalase activity displaced a slight shift in decrease. Chen et al. (2012) and Gao et al. (2010) discovered that soil catalase activities decline with the increasing availability of heavy metals, which as per the outcomes of this research as shown in Figure 4 as conditions remain constant for many days. The catalase activity does, however, increase as the day progresses. On (10, 20, 30, 45) days, (0.0, 0.1, 1.0) mg/L concentrations had a separate reading of a rise and decrease in catalase activity as the days pass.

This may have been due to the enzymes' activities having too much energy to withstand the cell's pressure from the low concentration of Cd as heavy metal. As the low levels of Cd concentration remain constant, respiration and other enzymatic activities begin to take place within the cells of the enzymes, reducing the impact of low Cd levels. Catalase enzyme activity rises with declining extractable heavy metal concentrations, over time through direct and indirect mechanisms (Cui et al., 2013b; Hu et al., 2014; Papa et al., 2010; Yang et al., 2016).

For example, (Ahmad et al., 2012) discovered a negative link between enzyme activities and lead (Pb) concentrations, as well as a similar showing of low Cd

concentration with catalase activity and other enzymes Hu et al. (2014). In this study catalase, activity showed no significant correlation with the concentration of Cd with (0.0, 0.1, and 1.0) mg/L extractable heavy metals. (Hu et al., 2014) also observed that catalase, urease, and acid phosphatase activity was only slightly affected by heavy metal pollution when concentration was low.

3.2. Effect of Cadmium on Urease Enzyme Activity of the Experiment

Cadmium concentration with Urease activity

According to Fernandes et al. (2005) in reviewing Brookes, 1995, The influence of trace metals on soil microbial activities has been reviewed extensively as suggested by (Moreno et al., 2001) on urease enzyme activity. Urease activity in soil has the capacity of the enzyme to catalyze the transformation of urea into ammonium (Kandeler et al., 1999). And play a critical role in the global nitrogen cycle's mineralization step, catalyzing the rapid hydrolytic decomposition of urea to produce ammonia and carbamate, the latter of which decomposes spontaneously into a second molecule of ammonia and bicarbonate (Blakeley et al., 1969; Mazzei et al., 2020).

Urease activity (0.58, 0.59, 0.55, 0.49, 0.41, 0.19) was inhibited in soils at the maximum Cd concentration levels (0 0.1, 1, 2, 5, 10) with urease activity (0.58, 0.59, 0.55, 0.49, 0.41, 0.19). High Cd levels demonstrated extreme toxicity against microorganisms, with rising Cd concentrations lowering urease activity.

From Figure 3, ammonia formed by the decomposition of urea into a second molecule of ammonia and bicarbonate in 24 hours was used to determine Urease activity (Blakeley et al., 1969; Mazzei et al., 2020), making Cadmium reaction effective on its activity. Higher levels of ammonia indicated higher urease activity, but an increase in Cd concentration affects Urease's respiration process, resulting in a gradual decrease in urease activity in the soil (Doelman & Haanstra, 1986; Zheng et al., 2019). Since the sample with zero (0) concentration remains constant through the experiment days, there is a consistent decrease in urease activity in the soil samples, which usually suggests a diminishing trend with rising Cd concentration from day 1 of culturing as shown in the graph.

According to Doelman & Haanstra (1986), after 6 weeks of incubation, there was a required increase in Cd concentration to induce a 10% decrease in urease activity, confirming that urease activity decreased dramatically from 0.58 mg/g to 0.19 mg/g as concentration increased from 0 to 10.0 mg/L. Urease behaviours were found to be sensitive to the heavy metal Cd's inhibitory effect. Decreased enzyme synthesis is associated with restricted microbial growth due to loss of energy to repair cells rather than direct metal inhibition due to enzyme activities of biomass C in Cd-treated soils.

Cadmium concentration with time (days) on Urease activity

Mulvaney & Bremner (1978) stated that Urease has been extensively studied about its inhibition by heavy metal as reported by Nor (1982). (Bremner & Douglas, 1971) reported inhibition percentages of less than 5% in two air-dried

soils by adding 50 mg·kg⁻¹, Cr3, Ni, and Pb, while Cu caused inhibition of 13% - 16% (Tabatabai, 1977). The data on various concentrations of Cd with time and days on urease activity can be understood as the experiment takes in 45 days of this study. With Figure 4, the readings the sample with no Cd concentration or 0.0 g/mol serving as the control experiment had little slight changes of reading with each day unfolding.

The various concentrations of Cd have a particular effect on urease function, as shown graphically in Figure 4. On day 10, soil urease activity in the soil showed a decreasing trend as Cd concentrations increased. Since the condition was not greatly affected by high concentration, urease activity was greater in the soil at a concentration of 0.0 mg/L than at a concentration of 10.mg/L. According to (Zantua & Bremner, 1975, 1977) urease activities and microbial biomass remain constant under these conditions for a very long time as reported by Nannipieri et al. (1983). Cd had a significant effect on urease activity at higher concentrations. Toxicity of Cd greatly reduced microbial abundance thus stimulating enzyme respiration. Regarding Moreno et al. (2001), the decrease in urease activity content or inhibition by Cd is due to the heavy metal's adverse effects on the activity of microbial species susceptible to Cd emissions reflecting on 10.0 mg/L.

From day 10 to day 45, the concentrations of Cd 0.1 mg/L and 1.0 mg/L increase and decrease, indicating enzymatic activity. The increase and decrease of the readings shown in the graph indicate exhibiting a rise in energy to withstand the effect of Cd on enzymatic activity by a transformation of nitrogen in the soil (Adetunji et al., 2017; Makoi & Ndakidemi, 2008). A high concentration of Cd (10.0 mg/L) had a significant impact on urease activity, putting additional strain on cell activities. This result corresponds to the outcome by Shi & Ma (2017), who also indicates a report from Meng et al. (2018), discovering that mass fraction of heavy metals at low concentration, may enhance soil enzyme activity; a high mass fraction, influence soil enzyme activity excessively.

Aside from the long-term efficacy of Cd concentration on Urease production, an optimal stabilization of low concentrations has no major negative effects on the biological status of the soil (O'Connor et al., 2018; Tang et al., 2020). These results were in line with previous studies that reported that heavy metal content may affect soil enzyme activity by direct and indirect mechanisms (Cui et al., 2013b; Hu et al., 2014; Papa et al., 2010; Yang et al., 2016). For instance, stated by Ahmad et al. (2012), as reflected in Figure 4.

3.3. Effect of Cadmium on Dehydrogenase Enzyme Activity

Dehydrogenase enzymes transfer protons and electrons from substrates to accepters, which aids in the biological oxidation of soil organic matter (Tabatabai, 1982). DHA act as an indicator of microbial oxidative activities and microbiological redox systems (Dick et al., 1997; Von Mersi & Schinner, 1991). Investigations by (Stevenson, 1959) have also shown, for certain Canadian soils, has is a strong as-

sociation (r = 0.84) between the reduction of 2,3,5,-triphenyltetrazoliumchloride (TTC) and O_2 absorption (r = 0.84) (Tabatabai, 1994) soils.

Cadmium concentration with dehydrogenase activity

Soil dehydrogenase activity correlates with biological activity and microbial communities in the soil. Intracellular dehydrogenase enzymes are oxidoreductases that catalyze organic compound oxidation by splitting two H atoms making Dehydrogenase activity a strong predictor of microbial oxidative activities in soils and it is also used for indicating microbiological redox systems (Tabatabai, 1982). Dehydrogenase activity (48.0, 53.0, 59.7, 63.5, 52.6, 38.7) was inhibited in soils at the maximum Cd concentrations (0 0.1, 1, 2, 5, 10) with dehydrogenase activity (48.0, 53.0, 59.7, 63.5, 52.6, 38.7). High Cd levels exhibited extreme toxicity against microorganisms as Cd concentrations increased, lowering dehydrogenase activity in the soil.

China in 2014, recorded 10% to 20% of its soil been contaminated by heavy metals (Ye et al., 2014), of which Cd contamination being the most serious issues in arable land according to the Bulletin on National Survey of Soil Contamination, jointly issued in 2014 by the Ministry of Environmental Protection of China and Ministry of Land Resources of China. Dick, 1994; Dick et al. (1997) checked the effect of Cadmium on the soil microbial activity and at what concentration of Cd is considered to have more influence on dehydrogenase activity as shown in this study. Malley et al. (2006) discovered an overall reduction in dehydrogenase activity. Nweke et al. (2007) concluded that for all the metal ions (Cd^{2+}) there was progressive inhibition in dehydrogenase activity and rhizoplane microbial community with each successive increase in the concentration of metal ions. Cd had a strong active effect on the size of soil microbial biomass and Dehydrogenase enzyme activities.

From Figure 5, dehydrogenase activity increases with low concentration from (0.0, 0.1, 1.0, 2.0) mg/L with increase in the dehydrogenase activity (49.8, 53.4, 59.7) mg/kg. d as DHA releases energy to repair damaged cells by Cd. The readings show that a low concentration of Cd does not have a major significant effect on dehydrogenase activity. Interestingly the dehydrogenase activity shows an increase with increasing concentration of Cd of (0.1, 0, 2.0) mg/L to dehydrogenase activity (52.6, 38.7) mg/kg.d. Dar (1996) reported a decrease in dehydrogenase activity (DHA) and alkaline phosphatase activity at 50 mg Cd kg in a laboratory study with different soil types.

Landi et al. (2000) found a negative effect of Cd on DHA, but at a much higher concentration of 500 mg Cd kg whereas dehydrogenase and acid phosphatase activity decreased at 50 mg Cd kg. The Cd concentration required to cause a similar inhibitory effect on phosphatase activity and DHA was 10 times greater in clay soil than in sandy soil (Doelman & Haanstra, 1989). With this study, the Cd concentration needed to cause a similar inhibitory effect on dehydrogenase activity was 10.0 mg/L greater than its effect.

Cadmium concentration with time on dehydrogenase activity

Concentration of (0.0. 0.1, 1.0 10.0) g/mol in 0 to 45 days had their respective

data readings on the dehydrogenase activity. Figure 6; below gives the readings of the sample with no Cd concentration or 0.0 g/mol which serves as the control experiment that had slight changes of reading with each day unfolding.

Each data collected gives a clear definition of the influence of cadmium on the various enzymatic activities. Cd toxicity increased with the incubation time; therefore, the lowest microbial biomass and enzyme activity was observed at forty-five days of incubation. The results revealed that soil microbial biomass and enzyme activities were strongly inhibited by Cd. Rogers & Li (1985). According to the graph, dehydrogenase activity increases with low concentrations from (0.0, 0.1, 1.0) mg/L, Tan et al. (2017) with a rise in dehydrogenase activity on (0 - 45) days with Cadmium incubation for days, low concentration of Cd does not have a major significant effect on dehydrogenase activity. Although the concentration of Cd rises from 0.0 to 1.0 mg/L, which was supposed to negatively affect the Dehydrogenase activity, the respiratory soil properties transform to increase the dehydrogenase activity (Zheng et al., 2019).

Cd concentrations were increased, but the magnitudes of the decreases were not proportional to the Cd concentration added (Alef & Kleiner, 1986; Casida, 1977; Tabatabai & Bremner, 1969) as shown in Figure 6. Also in sludge amended soils, high Cd concentrations inhibited dehydrogenase, alkaline phosphatase, and arginine-ammonification activities, while low Cd concentrations increased dehydrogenase operation (Effron et al., 2004). The toxicity of Cd dehydrogenase activities decreased in soil with low pH (4.8) and high organic carbon (2.3%) (Moreno et al., 2001). In addition, the basic enzymatic activities are striking and can be used to distinguish variations in dehydrogenase activity between the Cadmium concentrations (Tan et al., 2017; Tang et al., 2020; Xin et al., 2017).

3.4. Comparing the Enzymatic Activities with Cadmium

Comparably, the activities of urease, dehydrogenase and catalase differed significantly between treatments in this study, and the enzyme activities decreased as heavy metal concentrations increased. Heavy metals inhibited urease and dehydrogenase activity in general, supporting the findings of previous research (Wiatrowska et al., 2015; Xian et al., 2015). Other researchers explained, soil enzyme activities decrease with the increasing availability of heavy metals, Xian et al. (2015). Similarly, it was shown that Cd had a distinct inhibitory effect on urease, acid phosphatase, and catalase activities (Mao et al., 2015; Xin et al., 2017).

According to our findings, the order of sensitivity enzymes in response to Cadmium contamination is catalase > urease > dehydrogenase. Catalase activity decreased even when Cd levels were low, followed by Urease activity, and eventually dehydrogenase activity. The microbial community structure was heavily influenced in this study, which was consistent with lower microbial activities at various levels of Cd concentration and time. Furthermore, there was a significant community shift in this study, especially at high levels of Cd contamination, which is likely due to metal toxicity as well as nutrient scarcity because no nu-

trients were provided during the incubation period (Khan et al., 2010).

The activities of Catalase, Urease, and Dehydrogenase were all affected by Cd contamination. Each soil enzyme is sensitive to heavy metals in a different way Sethi & Gupta (2015); Shen et al. (2005); Shen et al. (2005). Urease and dehydrogenase can be useful indicators of combined pollution heavy metals, particularly in the early stages of pollution, according to a study, Sethi & Gupta (2015), Yang et al. (2016). Our findings for soil respiration may be used to estimate the capacity for heavy metals in polluted field soils to adversely impact soil microbial group responses, and soil respiration activity was close to that of the microbe population under Cd.

4. Conclusion

It is well known that cadmium emission reduces the biological activity of soil microorganisms. Soil enzyme activity may be used as a metric to measure the impact of heavy metals on biological activity in the soil. In soil, catalase and urease are sensitive to heavy metals, including Cd, and can represent heavy metal toxicity on soil microorganisms. Enzymatic activity declines have been linked to soil metal load by the majority of researchers. Also, different soil enzymes responded differently to elevated heavy metal concentrations. Soils with the highest concentrations of Cd had the lowest levels of soil enzyme activity.

As a result, decreases in microbial populations have been reported in the soils polluted with heavy metal compounds. In addition, heavy metal pollution can inhibit or even destroy sensitive soil microorganisms, associated with decreased soil respiration and enzyme activity with increasing Cadmium levels. Gradual changes in enzymatic community composition were observed in this study as a result of Cd gradients, suggesting the existence of dose-related effects, as previously stated.

Heavy metals can inhibit enzyme activity by interfering with the enzyme-substrate complex, denaturing the enzyme protein, and interfering with the active sites, or by interfering with the microbial cells' ability to synthesize enzymes. Changes in the group structure caused by metals may also affect enzyme activity. Heavy metal toxicity induced the death of a large number of microorganisms, and the surviving microorganisms required energy supply for survival and resistance to heavy metal toxicity.

Enzymes including Catalase, Urease, Dehydrogenase, and Alkaline Phosphatase, as well as other enzymatic activities, are inactivated when Cd binds to active sites, causing metabolism to be disrupted. Cadmium, in addition to being an enzyme inhibitor, can harm membrane structure and function by binding to ligands including phosphate and protein cysteinyl and histidyl groups.

The following conclusion of Cd in relation with time, concentration among these enzyme activities (Catalase, Urease, and Dehydrogenase) are listed below:
- High Cd concentrations influenced the activity of soil enzymes catalase, urease, and dehydrogenase. When Cd concentrations were poor, the total

metal content and enzyme activity had a weak effect on the enzymes.

- There are significant variations in the activity of Catalase, Urease, and Dehydrogenase enzymes, with dehydrogenase activity increasing with lower Cd concentrations.

- Cadmium is classified as a class 1 human carcinogen, although a weak genotoxic has been identified as a hazardous element contaminate to soil enzyme activities by various researchers. As a result, the enzymes studied in this study can be grouped according to their sensitivity to Cd: catalase > urease > dehydrogenases.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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