Effect of various pollutants

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Abstract

Soil is a natural resource that is utmost importance from an agricultural as well as environmental perspective. Good soil health not only helps improve the quality and yield of agriculture products, but is also important for recycling of significant amounts of organic biomass. However, pollution of soil by various pollutants, such as heavy metals, pesticides or petroleum hydrocarbons, can lead to serious environmental as well as biological effects. This pollution can directly harm animals and human being, leach into drinking water source, damage plants and vegetation, as well as affect the endemic microorganisms and insects living in the soil.

Microorganisms that are normally found in the top soil play key roles in enzymatic recycling of nitrogen, phosphorous, and sulfur, as well as the decomposition of organic matter; thus they impact global recycling of nutrients, carbon and other elements. In addition to nutrient recycling, soil microbes can also affect physical properties of soil as they secrete extra-cellular polysaccharides which stabilize soil aggregates, which in turn affect water retention, infiltration rate, crusting, erodibility of soil. Thus, microbial health is considered a good indicator of soil health. Heavy metal pollutants found in the soil con cause their deleterious effects by one of four ways: 1) These heavy metals can undergo redox cycling and in the process lead to oxyradical production which then causes oxidative stress in organisms, 2) they can bind directly to sulfhydryl groups of proteins and lead to protein inactivation and denaturation, 3) they can bind to intracellular glutathione (GSH) or antioxidant enzymes (e. g. SOD, Catalase, GSH reductase/ peroxideaes) and reduce the antioxidant ability of cells; and 4) these heavy metals can also compete for metal-cofactor binding of metallo-enzymes and lead to their inactivation. Recent work on the effect heavy metal pollution on soil have shown that like microorganisms, activity of the enzyme (produced by microorganisms), can also be used as indicators of soil health. The soil enzymes that have been used for these studies are dehydrogenase, beta-glucosidase, cellulose, phenol oxidase, urease, amidase, phosphatase and arylsulphatase. Most of these studies have been carried out by monitoring enzyme activities of pollutant treated soil. Though a valid approach, the pollutants in these studies could have a systematic effect on the microorganism and so that the decreased or increased soil enzyme activity could be due to other reasons, and not due to the direct effect of the pollutant on various enzymes. Additionally the tested pollutants could be metabolized by the microorganisms or broken down by the environmental factors, and so their effect on microbial enzymes could be secondary product. A few studies have been published in which the effect of heavy metal pollutants have been examined on purified enzymes in vitro, however they are very few and not very thorough. In this thesis, we have systematically examined the effect of the various of heavy enzymes such as (Mg+2, Ca+2, Co+2, Cd+2, Hg+2, Cu+2) on alkaline phosphatase (ALP) activity, and show that the heavy metals such as Hg+2, Cu+2 inhibited the enzyme more strongly than Cd+2, and Co+2 . However, the positive bivalent alkaline-earth metals such as Ca+2, and Mg+2 activated the enzyme, with Mg+2 being a stronger activator of the enzyme than Ca+2. Also, the monovalent alkali metal ions such as Na+ had no effect on the ALP Activity. We also examined the effect of combining heavy metals on ALP activity; such as Ca+2 + Cd+2, Ca+2 + Hg+2, and Cu+2 + Hg+2.

In the second part of the study, we examined the effect of oxy-radicals such as those generated by UV/H2O2 on the ALP activity. We also looked at the effect of UV together with heavy metals on ALP activity. We observed that UV radiation by itself did not affect the enzyme, however the combination of UV and Cu+2, Hg+2 or Cd+2 inhibited the activity of the enzyme more than when the heavy metals were incubated by themselves alone. Moreover, H2O2 alone or together with heavy metals significantly inhibited the ALP activity as well.

The data presented here show that the important bacterial enzyme, alkaline phosphatase is very sensitive to heavy metal exposure and is readily inactivated by them. Furthermore, we show that conditions that lead to oxyradical production increase the extent of enzyme inactivation by various heavy metals. In summary, we show that heavy metal pollution is of serious concern for good soil microbial health, which has the potential to affect agriculture and food production directly

Key words: Bacterial alkaline phosphatase, heavy metal, pollution, soil, microorganisms, mercury, cadmium, cobalt, copper, calcium, oxyradicals, UV radiation, hydrogen peroxide.

Chapter I

INTRODUCTION

Environment and pollution

Environment is classically defined as the natural world of land, sea, air, plants, and animals [1] and is therefore directly affected by various factors. With the advent of the industrial revolution in the 19th century and the discovery of oil, our natural environment is constantly being affected by human as well as non-human activities. By definition, " pollution" is the introduction of contaminants into the environment that cause harm to humans, other living organisms, or damage the environment. Pollution can arise from chemical substances, energy (such as noise or heat) or even light. The sources of pollution can be natural causes, for example through volcanic eruptions, or man-made, such as car exhaust emissions [2, 3]. When these polluting agents directly affect the environment, the term " environmental pollution" is used and is defined as contamination of air, water and land from man-made waste [2].

Environmental pollution can be further divided into:

- Air pollution,
- Water pollution, and
- Soil pollution.

Air can be contaminated by volatile organic compounds (VOC), acid rain (produced from sulfur dioxide and nitrogen dioxide combining with water), as well as airborne particles [4].

Water can be contaminated by herbicides, food processing wastes, volatile organic compounds (VOC), pesticides, heavy metals, and other chemical wastes [5].

Lastly, soil can be contaminated by various agents, such as petroleum-based hydrocarbons, factory-generated chemicals, pesticides, as well as heavy metals [2].

Soil pollution

Soil pollution is broadly defined as the introduction of substances, biological organisms, or energy into the soil, such as chemicals, salts, radioactive materials, or disease causing agent resulting in a change of the soil quality, which is likely to affect the normal use of the soil or endangering public health and the living environment [4].

From LoveToKnow Garden

There are many different sources for soil pollution such as, seepage from a landfill, discharge of industrial waste into the soil, leakage of contaminated water into the soil, leakage of underground storage tanks, excess application of pesticides, herbicides or fertilizer and solid waste seepage [6]. However, the most common chemicals that cause soil pollution are: petroleum hydrocarbons, heavy metals, pesticides, organic solvents, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated aromatic compounds, as well as inorganic compounds, such as nitrates, phosphates, inorganic acids and radioactive substances [7].

Soil is an extremely important resource for human beings, not only because it impacts our agriculture directly, but also since it affects domestic animals that are crucial to our survival. As would be expected, good soil health improves the productivity of agricultural crops. By definition, soil health is the capacity of soil to function as a vital living system, within an ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environment, and maintain plant, animal, and human health [6-7].

The importance of soil is further highlighted by the fact that soil works as an environmental filter to remove various toxic substances [7]. It is also worth pointing out that since regeneration of soil by chemical and biological processes takes a very long time, soil is considered to be a very precious, non-renewable resource. it is believed that one components of soil that is most susceptible to soil pollution are the microorganisms that are present in soil [8].

The microorganisms in soil

The surface layers of soil contain the highest numbers and variety of microorganisms [10]. These microorganisms in soil play the important role of breaking down both, organic and mineral materials partly by enzymatic action and partly by taking the materials in as nutrients and metabolizing them further. Most of the breakdown products are used by the plants as nutrients or are lost into the soil environment. However, the rest of these breakdown materials are used to generate humus. Humus plays an active role to further enhance soil property, e. g. it improves the water-holding capacity of soil which makes more water available to the plants, a supply plant with nutrients, increases the adsorption of minerals, and contributes to soil aggregation [9]. Moreover humus increases the soil buffering capacity and stabilizes the soil's pH to further help the plants to take up mineral nutrients. Additionally, it darkens the soil color which increases the soil's absorption of solar energy [10].

In addition, the decomposition of the plant and animal tissues by microorganisms can recycle energy and convert nutrients to forms that are usable for plants. Also, very importantly, microorganisms play vital roles in cycling of sulfur, phosphorus, iron, and many micronutrient trace elements [11]. The transformations of elements to various forms are described as cycle. In the carbon cycle, microorganisms transform plant and animal residues into carbon dioxide and the soil organic matter known as humus. In the nitrogen cycle, nitrogen is made available to plants only when it is transformed to ammonia (NH3) either by soil bacteria such as (N2 fixation) or by humans such as (manufacture of fertilizers) Soil bacteria also can return the nitrogen to the atmosphere by transforming NO3- to N2 or (N2O) gas [12].

Another very important element found in soil is Phosphorus, which is critical for improving the soil fertility [13-14], thus increasing the forest and agriculture production. As shown in fig: 1. 1 Phosphorus is usually found in rocks and it becomes available for plant after weathering and dissolving in the soil water. It is then absorbed by plants and then gets transferred to animals, the animal and plant residues containing this phosphorus can be recycled again by microorganisms. Additionally, fertilizers are also an important source of phosphorus in the soil [18-20].

As can be seen, microbial enzymes play critical and crucial roles in recycling of important elements as shown in table 1 [12, 13]. In addition to recycling carbon, nitrogen, and phosphorus, microbial enzymes play critical roles in the recycling of sulfur and other nutrients, Hence microbial enzymes are critical for good soil health. Due to this reason, it is well established that microbial enzyme activity is a very good indicator of the health and quality of soil. Therefore, factors that affect microbes or microbial enzymes, such as pesticides or heavy metals, will inevitably lead to affecting the quality of soil [19-20]

Alkaline phosphatase (ALP)

As mentioned above, most of the phosphorus recycling in soil is due to the action of microbial enzyme on organic (plant/animal) matter. The enzyme responsible for this is Alkaline Phosphatase (ALP). ALP is an enzyme that has the ability to remove the phosphate group from many types of molecules including proteins, nucleotides, and alkaloids – a process known as de-phosphorylation [22]. As the name suggests, ALP is more active under alkaline environment, for example the optimal pH for the activity of E. coli theenzyme ranges from 8 – 9. 5 [23]

Bacterial ALP

Bacterial ALP is located in the periplasmic space, external to cell membrane and this space is affect by the environment more than the actual interior of the cell. Bacterial ALP is a very robust enzyme as it known to be resistant to inactivation, denaturation, and degradation by various chemicals and denaturants [15].

In addition, ALP is produced only by bacteria only when it is needed, such as during phosphate starvation and not when the phosphate is plentiful. Although the exact physiological function of bacterial ALP is not known, it is hypothesized that this enzyme is needed for free phosphate generation for uptake and use. However, it is also possible that ALP-mediated dephosphorylation maybe important for efficient uptake of various organic molecules (which is normally prevented by the presence of phosphate groups on these molecules) [22-25].

General structure of E. coli alkaline phosphatase

The structure of ALP is composed of two identical subunits each containing 471 amino acids, fig. 1. 2 [24]. ALP also has four Cys residues that are present as two intra-chain disulfides (190-200) & (308-358), fig. 1. 3. The active site of alkaline phosphatase contains essential metals ions – two zinc and one magnesium ions. The magnesium ion is coordinated by Asp73, Asp175, Thr 177, Glu 344 [27]. The zinc ions are coordinated by Asp 73, Asp349, His 353, Asp391, His 392, His 343 as well as a water molecule, fig. 1. 3 [26].

Human ALP

In human body the ALP is present in liver, bile duct, kidney, bond, and placenta. Like the bacterial enzyme, human ALP is also a dimer, however the optimal pH for the human ALP is = 10. In humane, measurement of ALP activity is used extensively in medical diagnostics clinical setting [30]

The importance of measuring alkaline phosphatase is to check for the possibility of bone disease or liver disease. Increase serum alkaline phosphatase can be either due to quick growth of bone; because of it's produced by bone-forming cells. Or due to improper function of liver [31]. The amount of the ALP in the blood is dependent on the age, as children in the growing stage have much more ALP than the adults. In various states elevating of the ALP is more common than decreased. Elevating level of ALP is indicating liver disease, bone disease, some drugs (carbamazepine, phenytoin), and pregnancy. Decreased level of ALP can be due to zinc deficiency, vitamin C deficiency, folic acid deficiency, excess Vitamin D intake, as well as Low phosphorus levels [32].

Heavy metals

Heavy metals innature are one of the important components in the Earth's crust. There are 23 elements that are classified as " heavy metals": and the most common being : antimony, arsenic, bismuth, cadmium, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, and zinc [33].

Small amount of these elements are usually found in our environment, but large amounts of any of them may cause acute or chronic toxicity (poisoning). Heavy metals can enter our bodies by many ways such as drinking water, food and air, however the biggest danger of heavy metal comes from bioaccumulation [34].

Heavy metal toxicity can damage the central nervous function, blood component, lungs, kidneys, liver, and other vital organs, as well as cause fatigue. Long-term exposure may result in slow and progressive physical, muscular, and neurological degeneration that mimic Alzheimer’s disease, and multiple sclerosis[35]. Heavy metals can enter our water supply by industrial and consumer waste release of heavy metals into streams, lakes, rivers, and underground water, as well as from acidic rain[34-35].

The effects of the heavy metals in the environment.

The Agency for Toxic Substances and Disease Registry in the United States (ATSDR) is responsible for assessment of waste sites and providing health information concerning hazardous substances, response to emergency release situations, and education and training concerning hazardous substances [36].

In cooperation with the U. S. Environmental Protection Agency, the ATSDR has compiled a priority List for 2001 called the “ Top 20 Hazardous Substances. It is worth noting that heavy metals are included in that list: arsenic (1), lead (2), mercury (3), and cadmium (7) [40, 41]. this further underscores the hazard posed by this metal pollution [35, 36].

Arsenic

Arsenic occurs naturally such as in air, rocks, water, and soil. It’s used a lot in industry such as wood preservative (accounting for 90%of it’s usage), but arsenic is also used in paints, dyes, metals, drugs, soaps and semiconductors. High arsenic levels can also come from certain fertilizers and animal feeding operations. Industry practices such as copper smelting, mining and coal burning also contribute to arsenic in our environment [36].

Arsenic exposure has both long-term and short-term effects. Shore-term effects include sore throat, skin rash, irritation and warts, numbness in hands and feet, diarrhea [37]. Long- term or chronic exposure to arsenic has been linked to cancer of bladder, lungs, skin, kidneys, and nasal passages, liver and prostate [38-39].

Chromium

Chromium is naturally found in rocks, soil, and volcanic dust and gases. It also used in metal alloys and pigments for paints, cement, paper, and rubber. Smaller amounts are used in drilling, textiles, and toner for copying machines. Chromium also often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium [40].

The low-level exposure to chromium can irritate the skin, cause shortness of breath, and coughing; however the long-term exposure can cause kidney and liver damage, and damage the circulatory system and nerve tissue. [41].

Cadmium

Cadmium is a byproduct of the mining and smelting of lead and zinc, also it is used in nickel-cadmium batteries, PVC plastics, and paint pigments; additionally it can be introduced in the environment through fertilizer, insecticide, and fungicide [42].

Short-term exposure to cadmium ca cause nausea, vomiting, diarrhea, muscle cramps, salivation, sensory disturbances, liver injury, convulsions, shock and renal failure. Where as, long-term exposure of the cadmium can cause, renal failure, swollen lung, cancer, and damage to kidney, liver, bone and blood [39].

When Cadmium is absorbed by an organism, it can remain resident for many years (over decades for humans) although it is eventually excreted, it can directly affect the human, plant and animal [43, 44]

Mercury

Mercury is one of the heavy metals. That is liquid at room temperature and therefore volatile. Additionally mercury not breaks down into less toxic substances easily due to its high density. When the mercury is discharged to the environment, it is always found in the bottoms of lakes and oceans. Depending on its chemical form, it may travel long distances before precipitation in fish, and water plants. Mercury is used in many industrial applications such as produce chlorine and caustic soda, in wiring devices and switches for electric lights, as well as, blood pressure monitors and thermometers. It is very toxic as even a few micro liters spilled on the skin can cause ill effects. In addition, microorganisms are able to convert the mercury to methyl mercury, which is readily absorbed by most organisms. The methyl mercury is bioaccumulation through the food chain [45]. Mercury can affect the plant by causes of growth inhibition or death of the plant, and that by changing of the membrane permeability of cell leading leakage of ions [44]. A mercury exposure may occur in the mining industry and in the manufacturing of fungicides, And as all heavy metals it have acute and chronic symptoms [47].

Acute exposure are cough, sore throat, and shortness of breath; metallic taste in the mouth, abdominal pain, nausea, vomiting and diarrhea; headaches, weakness, visual disturbances, tachycardia, and hypertension [39].

Chronic exposure to mercury may result in more severe and permanent damage to the central nervous system and kidneys. Mercury can also cross the placenta from the mother's body to the fetus (levels in the fetus are often double those in the mother) and accumulate, resulting in mental retardation, brain damage, cerebral palsy, blindness, seizures, and inability to speak [37],[48].

Copper

Copper is used as an electrical conductor in electrical wiring, in various metals alloy, as a thermal conductor and, in building materials [49] . Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth [50].

The short- term exposure of copper leads to severe vomiting, pain in the abdomen, and purging; followed by headache, and, in fatal cases, convulsions. Long -term effects of copper can cause weakness, with nervous restlessness; dizziness, cold sweats, and cramps, anemia, liver and kidney damage, and stomach and intestinal irritation, and eventually death [50, 51].

Iron

Iron is used mainly for steel and steel alloys, dyes, and abrasives. The strength of steel plays an active role in construction, including very tall buildings, and bridges with very wide spans. It has also been used in the manufacture of automobile bodies, ship hulls, and heavy machinery and machine parts. Additionally, the contamination of aquatic environment is often a result of drainage of iron contaminated acid from mining activities [39].

Iron can damage DNA, protein, lipids, and other cellular components, although cellular protein can bind and sequester free iron, but when this capacity is exceeded, free iron can react with peroxides to produce free radical. A process called " Fenton reaction"[52].

The short effects of exposure of iron are vomiting, cardiac depression, and irritation of nasal passages, throat, and lungs. [37].

Lead

Lead in the environment arises from both natural and anthropogenic sources. Exposure can occur through drinking water, food, air, soil and dust from old paint containing lead can also be found in batteries, petrol additives, alloys, pigments and compounds and cables.

Short-term effects, including elevation of blood pressure, reduction in the synthesis of hemoglobin, and decreased ability to utilize vitamin D and calcium [39]. High blood lead with increase exposure to lead, these problems become more severe. Levels in children are linked to low IQ and slow development [54]. Long term exposure can lead to damage of the central nervous system, with decreased mental functioning and hearing damage as two possible results. Furthermore, extensive liver and kidney damage can also occur, eventually leading to liver cancer [55-57].

Biochemical effect of heavy metals:

Biochemical (cellular) toxicology of heavy metals are attributed broadly to the following two mechanisms

1. Ability of the heavy metals to generate oxy-radicals and cause oxidation stress that can damage protein, nucleic acid, carbohydrate, and lipid membrane.
2. Ability of heavy metals to directory effect protein and enzymes, for example by binding to free sulfhydryl (thiol) groups of protein and affect their function. Moreover, some heavy metals can directly inhibit enzymes involved in GSH metabolism GSH synthetase and GSH reductase. Hg can also directly inhibit various free-radical quenching enzymes catalase, superoxide dismutase (SOD), and GSH peroxidase, thus leading to enhanced oxidative damage[59].

Oxyradicals and oxidative stress

Oxidative stress is defined as a condition in which production of oxyradical in the cells exceeds their ability to neutralize them. They are responsible for damaging proteins, nucleic acids, lipids and carbohydrates [57]. Moreover, they can also lead to many diseases such as cancer, and diabetes. Oxyradical such as O2-, H2O2, OH•, which are very strong oxidizing species can seriously affect all aspects cellular metabolism [60].

Most of the e. g. heavy metal as iron, copper, chromium, are capable of redox cycling, and in the cell, they can produce reactive radicals and can produce reactive oxygen species [61-63]. Therefore heavy metals that pollutants in the soil can affect the microorganisms by three possible ways: a) by producing oxyradical that cause oxidative stress on the organisms. b) Protein denaturation and direct enzyme, by binding directly to sulfhydryl group c) also heavy metals can compete for essential metals ions present in some metalloenzymes [63].

Objectives:

A few studies have been published in which the affect of the heavy metals pollutions have been examined on purified enzymes in vitro, however they are very few and not very through. In this study, our goal was to examine the effect of various heavy metals and oxy-radicals on the enzymatic activity of alkaline phosphatase, in vitro.

We had two specific objectives:

1. Examine the effect of four most common heavy metals pollutants on alkaline phosphatase activity in vitro. This was carried out by first optimizing the alkaline phosphatase assay and then examining the effect of Mg2+, Ca2+, Cd2+, Na+, Co2+, and Hg2+, either singly or combination, on ALP Activity.
2. Examine the effect of hydroxyl radicals on alkaline phosphatase activity in vitro, this was carried out by incubating ALP with H2O2 together with UV radiation or heavy metals to generate oxy-radicals.

Chapter II

MATERIALS AND METHODS

Materials and reagent

The following sections details the materials and methods used in the experiments outlined in this thesis.

All the chemical used including the tris buffer, p-nitro phenyl phosphate (? NPP), H_2O_2, and heavy metals ($HgCl_2$, $MgCl_2$, $CaCl_2$, $CdCl_2$, NaCl, $CoCl_2$ from Sigma Company. USA.

The alkaline phosphatase (ALP) was produced from Sorachem (France), (cat # LPP-219), with an activity of 36, 400 U? ml, (lot# 7135159A00),

Schematic of the experiment set-up

The assay was carried out as shown schematically in (fig. 2. 1). As can be seen in the figure, the reaction was carried out in a 4 ml cuvette, in tris buffer containing the substrate (pNPP). The reaction was started by adding the enzyme to the cuvette. The total volume of the reaction mixture was always 3ml.

As shown in fig 2. 2, the addition of the ALP resulted in the hydrolysis of pNPP to p-nitrophenol which under alkaline conditions absorbs at 405 nm.

The enzymes activity was calculated from the initial slope of this Abs vs. Time graph as shown in fig 2. 2.

Effect of heavy metals on ALP

To study the effect of heavy metals on ALP, various concentrations of chosen heavy metals were added to the buffer containing pNPP, prior to the addition of the enzyme. For experiments with two metals, they were both added to the buffer (with pNPP) together prior to the addition of ALP.

For experiment examining the effect of UV and heavy metals on ALP, the enzyme was incubated with 1mM of heavy metal and UV together for indicated time and then assayed for activity.

Effect of UV radiation on ALP

To study the effect of the UV on the ALP activity, ALP was exposed to UV light for (1, 5, 10, 15) minutes after which it was assayed for activity as previously described.

Effect of hydroxyl radicals on ALP

To study the effect incubation of the H2O2 and 1ml ALP under the UV light for 15 min and each (0, 1, 5, 10, 15)was take it and add to the cuvett that has chosen heavy metals with fixed concentration and buffer containing pNPP.

Unless otherwise mentioned, all the experiments were carried out in triplicates.

Optimization of ALP assay

Since an enzyme assay can be affected by various factors, we wanted to examine the three most important ones[pH, effect of substrate concentration, and effect of enzyme concentration]. And choose the optimum set of condition for all subsequent experiments.

Effect of pH on ALP activity

Figure 2. 3 show the activity of ALP in different pH buffers can be seen ALP had no measurable activity in pH= 6. 5 buffer, this is not surprising since it is known that ALP works best under alkaline conditions. The activity of the enzyme at pH= 7 was better, but not as good as when pH= 8. 5 or pH= 9. 5 was used.

Since the enzyme activity was the same in pH= 8. 5 and pH= 9. 5, we decided to use pH= 8. 5 buffer for all subsequent experiments.

Effect of substrate concentration (? NPP)

As can be seen from the figure 2. 4 the rate of ALP activity increased with increased in substrate concentration. This is consistent with basic enzyme kinetics that the initial velocity of an enzyme increase with increased substrate concentration until the maximum velocity(V max) is reached Although ? NPP concentration of 50 ? g/ml the fastest reaction in rate, we decided to use 33. 33 ? g/ml ? NPP for all experiment, to conserve the substrate being used

Effect of the enzyme concentration of ALP activity

ALP activity was measured at three different enzyme concentrations. As can be seen from figure2. 5, ofn three concentration analyzed 4. 5nM ALP give the bist result in term of the speed of the reaction.

Therefore, based on these optimization studies, we settled on using 50mM tris , pH= 8. 5 as the reaction buffer, 4. 5 nM of ALP, and 33. 33? g/ml as ? NPP as a standard set of conditions for all assays.

ALP_heavy metal effect_final figures_finalFigure 2. 5: Effect of ALP concentration on speed of ALP assay different amounts of ALP were incubation in pH= 8. 5 tris buffer with 33. 33ug/ml pNPP and incubated in pH 8. 5 tris buffer with various amounts of pNPP (substrate)and reaction was monitored as described under material and methods.

Reproducibility of the ALP assay

Lastly, we wanted to check to reproducibility of run our optimized ALP assay.

Chapter III

RESULTS AND DISCUSSION

The effect of the heavy metals on the ALP activity

The effect of the heavy metal on the ALP enzyme was studied, by adding a specified metal ion in to the assay mixture containing 50 mM Tris, pH 8. 5, 33. 33 ug/ml pNPP, and 4. 5 nM ALP for a in period of time (0, 5, 10, 20, 30) min. the activity (initial velocity, rate) of the enzymes was calculated by measuring increased absorption at 405nm [47] over time

The absorption was recorded by using spectrophotometer. Moreover the rate was obtained by the slop determining the slope of the linear portion of Abs 405 vs. time graph fig 2. 3

The enzyme activity = initial velocity = rate = Δ Abs / Δ time α Δ C / Δ time.

For comparing the initial