Effect of various heavy metals on the enzymatic activity of bacterial alkaline phosphatase

Maithah Maktoom Salmeen Al Nuaimi

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Effect of various heavy metals on the enzymatic activity of bacterial alkaline phosphatase

By

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2009-2010
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A thesis
Submitted to

United Arab Emirates University
In partial fulfilment of the requirements
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Supervisor

Dr. S. Salman Ashraf,
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Acting Dean, College of Science, **Prof. Mohammed N. Anwar**

United Arab Emirates University
2009/2010
Dedication

To my Father and Mother, I am proud to be your daughter!

Please always keep me in your thoughts and prayers.
Acknowledgement

I am deeply indebted to my supervisor Dr. Salman Ashraf, Associate Professor of Biochemistry, who gave me the honour to be one of his students. Also for his valuable advice and help throughout my whole thesis - from the experimental part to the reviewing of this thesis. I would also like to thank my thesis examiners Dr. Yusra Othman and Dr. Ahmed Almehdi – both of whom spent a lot of time and effort in reviewing and examining my thesis to further improve it. I truly appreciate all their hard work and help, especially Dr. Almehdi’s very thorough help!

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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 beings, 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 can 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 sulphhydryl 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/ peroxideae) 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 has 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 (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/H$_2$O$_2$ 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, H$_2$O$_2$ 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 could be 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.
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Figure 3.17: Effect of copper and hydrogen peroxide together on ALP activity
Abbreviations

ALP: Alkaline phosphatase.
pNPP: para-nitrophenol phosphate
H₂O₂: Hydrogen Peroxide
SD: Standard deviation.
Tris: Tris(hydroxymethyl)aminomethane
UV: Ultraviolet
SOD: Superoxide Dismutase
Hg: Mercury
Cu: Copper
Ca: Calcium.
Mg: Magnesium
Cd: Cadmium
Na: Sodium
Co: Cobalt
Vmax: Maximum velocity
Chapter I

INTRODUCTION
1.1 Environment and pollution

Environment is classically defined as the natural world of land, sea, air, plants, and animals\textsuperscript{[1]} and is therefore directly affected by various factors. With the advent of the industrial revolution in the 19\textsuperscript{th} 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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[4]}.

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

Lastly, soil can be contaminated by various agents, such as petroleum-based hydrocarbons, factory-generated chemicals, pesticides, as well as heavy metals\textsuperscript{[2]}.
1.2 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.\textsuperscript{14}

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.\textsuperscript{16} 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.\textsuperscript{17}

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.\textsuperscript{16-7}

The importance of soil is further highlighted by the fact that soil works as an environmental filter to remove various toxic substances.\textsuperscript{17} 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 component of soil that is most susceptible to soil pollution are the microorganisms that are present in soil. 

1.2.1 The microorganisms in soil

The surface layers of soil contain the highest numbers and variety of microorganisms. 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. 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.

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. 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 NO$_3^-$ to N$_2$ or (N$_2$O) 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$^{19,20}$.
Figure 1.1: Phosphorus cycle \[21\].
Table 1.1: Recycling of various elements by soil microbial enzymes.  

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<td>Cellulase</td>
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1.3 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* the enzyme ranges from 8 – 9.5 [23].

1.3.1 Bacterial ALP

Bacterial ALP is located in the periplasmic space, external to the cell membrane and this space is affected 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 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].
1.3.2 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. 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, Thr177, Glu344. The zinc ions are coordinated by Asp73, Asp349, His353, Asp391, His392, His343 as well as a water molecule, fig. 1.3.
Figure 1.2: Three-dimensional crystal structure of *E. coli* alkaline phosphatase (PDB id: 3bdg) \(^{27}\)
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Figure 1.3: Amino acid sequence and secondary structure of *E. coli* alkaline phosphatase$^{[29]}$
**Figure 1.4:** Metal-ion coordination and disulfide amino acids in *E. coli* alkaline phosphatase\textsuperscript{[29]}.
1.3.3 Human ALP

In human body the ALP is present in liver, kidney, bone, and placenta. Like the bacterial enzyme, human ALP is also a dimer, however the optimal pH for the human ALP is =10. In human, the 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 or liver disease. Increase serum alkaline phosphatase can be either due to quick growth of bone: because 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. Increase level of ALP is indicates liver disease, bone disease, physiologically. ALP is increased in children 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][33].
1.4 Heavy metals

Heavy metals in nature 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, gallium, iron, copper, lead, manganese, platinum, mercury, nickel, silver, tellurium, gold and zinc.\cite{35}

Small amounts of these elements are usually found in our environment, but large amounts of any of them may lead to acute or chronic toxicity. 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.\cite{35}

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.\cite{36} 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 acid rain.\cite{35-36}

1.5 The effects of the heavy metals on 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.\cite{37}

In cooperation with the U.S. Environmental Protection Agency, the ATSDR has generated a list of top 20 hazardous substances for 2001. It is worth noting that heavy metals are included in that list: arsenic is number 1, lead is in the second position,
mercury is at number 3 and cadmium at number 7\cite{18-39}. This further underscores the importance of studying heavy metal pollution\cite{36,37}.

1.6 Arsenic

Arsenic occurs naturally such as in air, rocks, water, and soil. It's has industrial usage such as wood preservation (accounting for 90\% of its usage), but arsenic is also used in paints, dyes, metals, drugs, soaps and semi-conductors. 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\cite{40}.

Arsenic exposure has both long-term and short-term effects. Short-term effects include sore throat, skin rash, irritation and warts, numbness in hands and feet, diarrhea\cite{38}. Long-term or chronic exposure to arsenic has been linked to cancer of bladder, lungs, skin, kidneys, and nasal passages, liver and prostate\cite{39-40}. 

15
1.7 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\[^{41}\].

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.\[^{42}\].

1.8 Cadmium

Cadmium is a byproduct of the mining and smelting of zinc and lead, also it is used in nickel-cadmium batteries, paint pigments; and PVC plastics additionally, it can be introduced in the environment through insecticide, fungicide, and fertilizer\[^{43}\].\[^{43}\].

Short-term exposure to cadmium can cause nausea, vomiting, diarrhea, muscle cramps, salivation, sensory disturbances, liver injury, convulsions, shock and renal failure. Long-term exposure of the cadmium can cause, renal failure, swollen lung, cancer, and damage to the kidney, liver, bone and blood\[^{40}\].

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\[^{44, 45}\].
1.9 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 [46]. 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 [45]. A mercury exposure may occur in the manufacturing of fungicides and in the mining industry and, And as all heavy metals it have acute and chronic symptoms [47].

Acute exposure can cause cough, sore throat, and shortness of breath; metallic taste in the mouth, abdominal pain, nausea, vomiting and diarrhea, weakness,; headaches visual disturbances, tachycardia, and hypertension [40].

Chronic exposure to mercury may result in more severe and permanent damage to the kidneys and central nervous system. Mercury can also pass from the mother’s body to the fetus throw the placenta and accumulate, resulting in mental retardation, seizures, brain damage, , blindness, cerebral palsy, and inability to speak [38],[48].
1.10 Copper

Copper is used as an electrical conductor in electrical wiring, in various metal 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, nervous restlessness, dizziness, cold sweats, and cramps, anemia, liver and kidney damage, and stomach and intestinal irritation, and eventually death\[^{50,51}\].

1.11 Iron

Iron is used mainly for steel making 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 radicals, this process called "Fenton reaction"\[^{52}\].
The short effects of exposure of iron are vomiting, cardiac depression, and irritation of nasal passages, throat, and lungs.\textsuperscript{[37]}

1.12 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. Lead can also be found in batteries, petrol additives, alloys, pigments and compounds and cables.

Short-term ill effects of lead exposure include elevation of blood pressure, reduction in hemoglobin synthesis, and reduced ability to use vitamin D and calcium\textsuperscript{[39]}. Very high levels of Pb in children are linked to low intelligence and slow development\textsuperscript{[54]}. Long term exposure can also lead to damage of the central nervous system. Furthermore, extensive liver and kidney damage can also occur, which may cause liver cancer\textsuperscript{[55-57]}. 

1.13 Cobalt

Cobalt is found naturally in the Earth’s crust most commonly in the form of arsenides and sulphides. Cobalt pollution results primarily due to its presence in our daily-use items, such as various alloys (e.g. Alnico), in Li-ion batteries, catalysts, and dyes and pigments\textsuperscript{[58]}. Cobalt is also found naturally as essential co-factors in various biomolecules, e.g. vitamin B12 and proteins\textsuperscript{[59]}. Although cobalt is not as toxic as other heavy metals, exposure to high levels of cobalt can have various ill health effects. Workers who have been exposed to high
levels of cobalt in the air have reported various lung-related conditions such as asthma and wheezing. Additionally, people who have ingested large amounts of cobalt have reported nausea and vomiting. Furthermore, there is some indication from animal studies that high levels of cobalt may harm the development of fetuses. There has been some link suggested between cobalt and cancer by the International Agency for Research on Cancer \cite{60}\ however, it appears that cobalt-induced cancer requires a direct contact between cobalt and skin/muscle and that air, food and water carried cobalt does not cause cancer in animals\cite{61}. 
<table>
<thead>
<tr>
<th>Metal</th>
<th>Acute symptoms</th>
<th>Chronic symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Nausea, vomiting, diarrhea, painful neuropathy</td>
<td>Diabetes, cancer: lung, bladder, skin,</td>
</tr>
<tr>
<td>Cadmium</td>
<td>nausea, vomiting, diarrhea, muscle cramps</td>
<td>Failure swelled lung cancer, Kidney, liver, bone and blood damage.</td>
</tr>
<tr>
<td>Chromium</td>
<td>irrate the skin, shortness of breath, coughing</td>
<td>Kidney and liver damage, and damage to circulatory and nerve tissue</td>
</tr>
<tr>
<td>Copper</td>
<td>severe vomiting, pain in the abdomen, and purging; afterwards headache</td>
<td>Anemia, liver and kidney damage, and stomach and intestinal irritation</td>
</tr>
<tr>
<td>Iron</td>
<td>Vomiting, cardiac depression, irritation of nasal passages, throat, and lungs.</td>
<td>iron pigmentation of the lungs</td>
</tr>
<tr>
<td>Lead</td>
<td>Nausea, vomiting headache</td>
<td>Encephalopathy, anemia, abdominal pain, nephropathy.</td>
</tr>
<tr>
<td>Mercury</td>
<td>Abdominal pain, weakness, nausea, vomiting and diarrhea; visual disturbances, tachycardia, headaches and hypertension.</td>
<td>Damage to the central nervous system and kidneys.</td>
</tr>
</tbody>
</table>
1.14 Biochemical effects 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 sulphydryl (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 directly inhibit various free-radical quenching enzymes catalase, superoxide dismutase (SOD), and GSH peroxidase, thus leading to enhanced oxidative damage[^63].

1.15 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. Oxyradicals such as $\mathrm{O}_2^-$, $\mathrm{H}_2\mathrm{O}_2$, OH', which are very strong oxidizing species can seriously affect all aspects cellular metabolism[^64].

Most heavy metals such as iron, copper, and chromium, are capable of redox cycling, and in the cell, they can produce reactive radicals which produce reactive oxygen species[^65-67]. Therefore heavy metals 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 enzyme, by binding
directly to sulphhydryl group c) also heavy metals can compete for essential metals
ions present in some metalloenzymes [66].
1.16 Objectives:

A few studies have been published in which the effect 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- To 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 Mg$^{2+}$, Ca$^{2+}$, Cd$^{2+}$, Na$^{+}$, Co$^{2+}$, and Hg$^{2+}$, either singly or combination, on ALP Activity.

2- To examine the effect of hydroxyl radicals on alkaline phosphatase activity *in vitro*. This was carried out by incubating ALP with heavy metals together with UV radiation or heavy metals with H$_2$O$_2$ to generate oxy-radicals.
Chapter II

MATERIALS AND METHODS
Chapter 2

MATERIALS AND METHODS

2.1 Materials and reagent

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

All the chemicals used including the Tris buffer, p-nitrophenyl phosphate (pNPP), H₂O₂, heavy metals (HgCl₂, CdCl₂, CoCl₂) and other reagents were from Sigma-Aldrich Chemical Company, USA.

The E. coli alkaline phosphatase (ALP) was purchased from Sorachem (France), (cat # LPP-219), with an activity of 36,400 U/ml, (lot# 7135159A00).

2.2 Schematic of the experimental set-up

The alkaline phosphatase assay used for this study was the standard assay based on the hydrolysis of the colorless p-nitrophenylphosphate (pNPP) to produce the yellow-colored phenolate anion, as initially reported by Sayer [68]. 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 and the absorbance at 405 nm (λ_max for phenolate anion) was measured using a spectrophotometer. 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 optimized assay conditions (which were used for all subsequent experiments) were as follows: 4.5 nM ALP in 50 mM Tris, pH 8.5, with 33.33 µg/ml pNPP at room temperature (about 22-24 °C).

The "enzyme activity" was determined by measuring the "initial velocity" of the enzyme, which in turn was calculated by measuring the "rate" of pNPP hydrolysis, which was obtained from the slope of the Abs (405 nm) vs. time graph (Fig. 2.2).

For comparing the initial velocity of the enzyme under different conditions, the results were expressed as relative to "no-treatment "ALP assay (normalized to 100%) and reported as "Normalized Rate". This normalization allowed us to compare the results obtained from different experiments.

2.3 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 (at equal concentrations) to the buffer (with pNPP) together prior to the addition of ALP.

2.4 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. For experiments examining the effect of UV and heavy metals together on ALP, the enzyme was incubated with 1mM of heavy metal and exposed to UV radiation (254
nm at a distance of 10 cm) for indicated time. At the selected time, 100 ul of the ALP (exposed to heavy metal and UV) was aliquoted out and added to a cuvette containing the Tris buffer and pNPP (33.33 µg/ml). The initial slope of the absorbance vs. time graph was calculated and used as a measure of the ALP activity.

2.5 Effect of hydrogen peroxide on ALP

To study the effect incubation of the $H_2O_2$ on ALP, the enzyme was incubated with (0.25 mM) $H_2O_2$ for specified time intervals at which point ALP was aliquoted out and added (final concentration of ALP 4.5 nM) to a cuvette containing 50 mM Tris (pH 8.5) and 33.33 ug/ml pNPP. The activity of the enzyme was measured from the slope of the linear portion of the Abs vs. time graph (as previously explained).

Similarly, for experiments involving hydrogen peroxide, 0.25 mM (final concentration) $H_2O_2$ and 1 mM heavy metals were incubated together with ALP for the specified time interval and then the ALP activity was assayed for activity.

The temperature for all the assays was the ambient room temperature, which was between 22-24 °C.

All the experiments were carried out at least in duplicates, and the results shown are the mean of replicates and standard deviation ($\sigma$). The standard deviation was calculated using MS Excel program using the following formula:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \mu)^2}{n-1}}$$

where $n = $ number of measurement, $\mu = $ mean and $x_i = $ ith value.
Coefficient of variation (CV) was also calculated to assess the quality of the data, as follows:

$$CV = \frac{\sigma}{\mu}$$

Where $\sigma$ is the standard deviation and $\mu$ is the mean of the data.
Figure 2.1: Schematic of the set-up of the experiment. As explained under Materials and Methods, 100 µl of 1 mg/ml pNPP (final concentration = 33.33 µg/ml) was added to 2.8 ml of 50 mM Tris, pH 8.5, buffer and a flat baseline was obtained. The ALP assay was started by adding 100 µl of 135 nM ALP enzyme (final concentration = 4.5 nM) and the change in color at 405 nm (due to hydrolysis of pNPP to produce yellow phenolate anion) was measured using a Cary 50 UV/Vis spectrophotometer.
Figure 2.2: Typical data obtained during an ALP assay. As explained in detail under Materials and Methods, the increase in absorbance at 405 nm (due to generation of the nitrophenolate anion by ALP – as shown in the reaction) was measured as a function of time and the slope from the linear portion of the curve was used to calculate the initial rate of ALP activity.
2.6. Optimization of ALP assay

Since an enzyme assay can be affected by various factors, we examined the three most important factors [pH, effect of substrate concentration, and effect of enzyme concentration]. We chose the optimum set of conditions for all subsequent experiments.

2.6.1 Effect of pH on ALP activity

Figure 2.3 shows the activity of ALP Tris buffer at different pH. As can be seen, ALP had no measurable activity in pH=6.5 buffers, 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.
Figure 2.3: ALP activity in different pH buffers. ALP (4.5nM) was incubated with 33.33ug/ml pNPP and the reaction was performed in different pH Tris buffer, as described under Materials and Methods.
2.6.2 Effect of substrate concentration (pNPP)

As can be seen from the figure 2.4 the rate of ALP activity increased with increase in substrate concentration. This is consistent with basic enzyme kinetics that the initial velocity of an enzyme increases with increased substrate concentration until the maximum velocity ($V_{max}$) is reached. Although pNPP concentration of 50 µg/ml had the fastest reaction in rate, we decided to use 33.33 µg/ml pNPP for all experiment, to conserve the substrate.
Figure 2.4: ALP activity as a function of substrate concentration. ALP (4.5nM) was incubated in pH 8.5 Tris buffer with increasing amounts of pNPP (substrate) and reaction was monitored as described under Materials and Methods.
2.6.3 Effect of the enzyme concentration of ALP activity

ALP activity was measured at three different enzyme concentrations. As can be seen from the figure 2.5. of three concentrations analyzed, 4.5nM ALP give the best result in term of the speed of the reaction.

Therefore, based on these optimization studies, we used 50mM Tris , pH=8.5 as the reaction buffer, 4.5 nM of ALP, and 33.33µg/ml pNPP at total volume of 3ml as standard set of conditions for all assays.
Figure 2.5: Effect of ALP concentration on the rate of ALP assay. Three different amounts of ALP (final concentrations of 2.25 nM, 4.5 nM, and 22.5 nM) were incubated in 50mM Tris buffer (pH 8.5) with 33.33ug/ml pNPP and reaction was monitored as described under Materials and Methods.
2.6.4 Reproducibility of the ALP assay

Finally, we wanted to check the reproducibility of our optimized ALP assay. As shown in figure 2.6, under our conditions the ALP assay was very reproducible most of the time, with coefficient of variation less than 1 for almost all the experiments.
Figure 2.6: Reproducibility of the enzyme assay. ALP (4.5nM) was added to 50mM Tris buffer, pH 8.5, with 33.33 μg/ml pNPP. The hydrolysis of pNPP was monitored at 405 nm as shown above for 4 minutes as described under Materials and Methods.
Chapter III

RESULTS AND DISCUSSION
3.2 The effect of the heavy metals on the ALP activity

Due to the obvious importance of ALP, a lot of studies have been published on this enzyme, including its inhibition by heavy metals\textsuperscript{[69-70]} However, most of these studies were carried out on whole soil\textsuperscript{[71]} or small organisms\textsuperscript{[72-73]} A few researches have looked at the effect of heavy metals on pure ALP \textit{(in vitro)}, however, these studies are either on immobilized ALP or ALP from non-soil bacterial source (green crab\textsuperscript{[74],[75]}).

Since these studies examine the effect of various heavy metals on \textit{E.coli} ALP are not widely reported in literature , we wanted to carry out a detailed study looking at how \(\text{Hg}^{2+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{and Cu}^{2+}\) would affect ALP.

The effect of the heavy metal on the ALP enzyme was studied by adding a specific metal ion to the assay mixture containing 50 mM Tris, pH 8.5, 33.33 \(\mu\)g/ml pNPP, and 4.5 nM ALP for a period of time (0, 5, 10, 20, or 30 min). The activity (initial velocity, rate) of the enzymes was calculated by measuring the slope of absorbance (405 nm) over time.

We wanted to examine the effect of as many heavy metals as possible on ALP activity. However, our initial data revealed that under our experiment condition and at high heavy metal concentration, many of the heavy metals we tested were not soluble (\(\text{FeCl}_2, \text{Pb(NO}_3\text{)}_2, \text{CrCl}_3, \text{Zn(NO}_3\text{)}_2, \text{Co(NO}_3\text{)}_2\)).
Heavy metals that remained soluble under our assay condition were: HgCl₂, CdCl₂, NaCl, and CoCl₂, we also included divalent metal ions such as Mg²⁺, and Ca²⁺ as positive controls in our study (Zn²⁺ could not be included since Zn(OH)₂ formed in alkaline buffers condition has extremely low solubility [76]. Additionally, we also tested the effect of high concentration of NaCl to show that our results were not due to ionic strength effect.

3.2.1 Effect of mercury on ALP Activity

Incubation of ALP with Hg⁺² led to pronounced decrease in enzyme activity. As seen in fig 3.1, increasing concentration of HgCl₂ in the assay buffer caused significant decrease in the rate of hydrolysis of the substrate pNPP (as seen by decreased slopes (rate) of Abs 405 vs. time graph (fig 3.1).

This is also shown in fig: 3.2& table 3.1, which shows, the initial rate of catalysis as a function of [Hg²⁺] concentration.

A similar effect has been previously published by Chen et al (2001)[74] when they examined the effect of Hg²⁺ on green crab ALP and Mazorra et al (2001)[73] when they examined the effect of Hg²⁺ on clams[73]. However, in their systems green crab and clam ALP seemed to be very sensitive to Hg²⁺. On the other hand, our *E. coli* ALP required higher concentration of Hg²⁺ to show inhibition. This difference could be due to fact that the enzymes in the three studies are from different organisms that live in different environments. Perhaps the bacterial ALP has evolved to withstand higher concentrations of heavy metals than higher organism (green crab[74] or clam[73]) ALP. This can be further tested and examined in detail in a later study.
Mercury is known to have a very high affinity for free thiol groups (cysteine) of protein. It is possible that Hg$^{2+}$ ions are binding to free thiol group of ALP, causing to ALP inactivation. Alternatively, it is possible than Hg$^{2+}$ can alter and distort the active site of ALP, or even compete for Zn$^{2+}$ and Mg$^{2+}$ binding sites in ALP.
Figure 3.1: Effect mercury chloride on ALP activity. As described under Materials and Methods, 4.5 nM ALP was incubated in 50 mM Tris (pH 8.5) buffer containing 33.33 μg/ml pNPP and increasing concentrations of HgCl₂. The increase in absorbance (corresponding to hydrolysis of pNPP and production of the phenolate anion) was monitored spectrophotometrically at 405 nm as described previously.
Figure 3.2: The effect of mercury on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 ug/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of HgCl$_2$ at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph (as shown in figures 2.2 and 3.1) and was normalized to the rate obtained without any HgCl$_2$ (control). Data shown is the mean and standard deviation of triplicates.
Table 3.1: The effect of increasing concentrations of mercury on ALP activity

<table>
<thead>
<tr>
<th>([\text{Hg}^{2+}], \text{mM})</th>
<th><strong>Normalized Rate</strong></th>
<th><strong>SD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
<td>13.75</td>
</tr>
<tr>
<td>1</td>
<td>78.41</td>
<td>7.71</td>
</tr>
<tr>
<td>5</td>
<td>52.33</td>
<td>2.79</td>
</tr>
<tr>
<td>10</td>
<td>41.77</td>
<td>6.04</td>
</tr>
<tr>
<td>20</td>
<td>11.78</td>
<td>7.00</td>
</tr>
<tr>
<td>30</td>
<td>2.66</td>
<td>3.13</td>
</tr>
</tbody>
</table>
3.2.2 Effect of the copper on ALP activity

Incubation of Cu$^{2+}$ with ALP also drastically inhibited ALP, very much like Hg$^{2+}$.

As can be seen from fig 3.3 and table 3.2, presence of CuCl$_2$ in the reaction buffer decreased the enzyme activity by up to 70%.

Again, these results are similar to what others have reported for ALP from different organisms – green crab [74]; clams (Mazorra et al., [73], and soil bacteria Wyszkowska, et al. 2005) [71].

The inhibitory effect of Cu$^{2+}$ seen here could be due to direct binding to ALP (like Hg$^{2+}$); however, due to the redox cycling nature of Cu$^{2+}$, it is possible that some redox reactions involving the enzyme may be involved as well.
Fig 3.3: Effect of copper on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 µg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CuCl₂ at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph (as shown in figures 2.2 and 3.1) and was normalized to the rate obtained without any CuCl₂ (control). Data shown is the mean and standard deviation of triplicates.
Table 3.2: Effect of copper on ALP activity

<table>
<thead>
<tr>
<th>[Cu^{2+}], mM</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>72.3</td>
<td>1.6</td>
</tr>
<tr>
<td>10</td>
<td>27.7</td>
<td>5.4</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>30</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>
3.2.3 Effect of cadmium on ALP activity

Treatment of ALP with Cadmium also led to enzyme inhibition (as measured by decrease in the rate of pNPP hydrolysis Fig 3.4, table 3.3). However, the inhibition caused by cadmium appears to be much less than that observed for Hg$^{2+}$ or Cu$^{2+}$.

This seems to indicate that perhaps Cd$^{2+}$ has a lower affinity for ALP than Hg$^{2+}$ or Cu$^{2+}$ and that the mode inhibition of ALP by Cd$^{2+}$ may be different than first two metals studied. Survey of published data show that there is some disagreement on the effect of Cd$^{2+}$ on ALP activity. Yang et al have reported inhibitory effect of Cd$^{2+}$ on soil bacterial ALP$^{76}$. Khan et al (2007)$^{77}$ also have reported a similar inhibitory effect of Cd$^{2+}$ on bacterial soil ALP activity, however Chen et al (2000)$^{74}$ show that green crab ALP was slightly (~ 20%) activated by Cd$^{2+}$. These differences could be due to the fact that Chen et al were looking at ALP from green crab, whereas ours (and Khan’s and Yang’s) ALP were of bacterial origin. Another study on non-bacterial ALP done by Mazorra et al found that Cd$^{2+}$ had different effect on ALP activities in different organs of clam$^{73}$. For example, they found that clam digestive gland ALP was not affected by Cd$^{2+}$ at all, whereas surprisingly ALP in the gills of clam showed very pronounced inhibitory effect. Additionally, Berezhetskyy et al have reported that immobilized bovine ALP was also inhibited by Cd$^{2+}$$^{75}$. These differences could be due to the slight differences in the amino acid sequences and tertiary structures of the different ALP studied by the various groups.

The mechanism of inhibition of ALP by Cd$^{2+}$ could be due to its binding the metal-ion binding site in ALP. This has been postulated by Poirier et al$^{72}$ who suggest that the inhibition of ALP by Cd and Cu could be due to these two element’s abilities to displace the native Zn$^{2+}$ or/ and disturb the enzyme 3D-structures.
Figure 3.4: Effect of cadmium ions on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CdCl₂ at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph (as shown in figure 2.2) and was normalized to the rate obtained without any CdCl₂ (control). Data shown is the mean and standard deviation of triplicates.
Table 3.3: Effect of cadmium ions on ALP activity

<table>
<thead>
<tr>
<th>$[\text{Cd}^{2+}]$, mM</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>30</td>
<td>52.7</td>
<td>14.9</td>
</tr>
</tbody>
</table>
3.2.4 Effect of cobalt on ALP activity

Incubation of ALP with cobalt showed a complex response. At low concentration, 
Co$^{2+}$ showed a significant activation of the enzyme ALP about 20% (table 3.4, and fig 3.5). However, additional amount of Co$^{2+}$ did not result in higher activation of the 
enzyme, but slower decrease back to control (no heavy metal added) level of ALP activity.

A similar activation of bacterial ALP (Bacillus sp.) has been reported by Spencer et al (1981)\cite{178} where they reported significant increase of ALP activity by 0.1 mM Co$^{2+}$ 
and Chen et al\cite{174}, when they also showed slight but significant activation of ALP by 
Co$^{2+}$.

This however, is in contrast to what Berezheskyy et al have reported in their study\cite{175} 
that Co$^{2+}$ was able to inhibit the activity of immobilized ALP by as little as 2ppm.

We believe the inhibitory effect of Co$^{2+}$ on ALP reported by Berezheskyy et al is due 
to the immobilization of the enzyme which is “locking” the enzyme in a non-ideal 
conformation and maybe an artifact of their system.
Figure 3.5: Effect of cobalt on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CoCl₂ at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any CoCl₂ (control). Data shown is the mean and standard deviation of triplicates.
Table 3.4: Effect of cobalt ions on ALP activity

<table>
<thead>
<tr>
<th>[Co$^{2+}$], mM</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>2.8</td>
</tr>
<tr>
<td>5</td>
<td>122.7</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>114.0</td>
<td>3.2</td>
</tr>
<tr>
<td>20</td>
<td>109.7</td>
<td>0.3</td>
</tr>
<tr>
<td>30</td>
<td>95.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
3.2.5 Effect of calcium on ALP activity

Study of the effect of positive bivalent alkaline earth metal ions such as Ca$^{2+}$ on the ALP activity shows concentration dependent activation of the enzyme. As can be seen from fig 3.6.. and table 3.5. that ALP activity is increased with increased the CaCl$_2$ concentration reaching the maximum 30% increase with 10-15 mM CaCl$_2$. additional Ca$^{2+}$ ions did not increase the activity any further. These results are similar to the previously published green crab ALP study [74] as well as by Brun et al. who showed activation of rat intestinal ALP activation by Ca$^{2+}$ [79]
Figure 3.6: Effect of Calcium on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CaCl₂ at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any CaCl₂ (control). Data shown is the mean and standard deviation of triplicates.
Table 3.5: Effect of calcium ions on ALP activity

<table>
<thead>
<tr>
<th>[Ca(^{2+}), mM]</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
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<td>6.9</td>
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<td>10</td>
<td>125.4</td>
<td>12.4</td>
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<td>20</td>
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</tr>
<tr>
<td>30</td>
<td>134.6</td>
<td>8.4</td>
</tr>
</tbody>
</table>
3.3 Summary of the effect of various heavy metals on the ALP activity

Figure 3.7 shows summarized result of the effect of 30 mM of various heavy metals on ALP activity. As can be seen, of the various metal ions tested only Hg$^{2+}$, Cu$^{2+}$, and Cd$^{2+}$ inhibited the ALP (Hg$^{2+}$ and Cu$^{2+}$ were most inhibitory, followed by Cd$^{2+}$). Sodium ions had about 20% inhibitory effect on the enzyme similar to that reported by Chen et al\cite{74}. where as divalent alkaline earth metals activated ALP, with Mg$^{2+}$ activation the enzyme the most, followed by Ca$^{2+}$. The activation of ALP by Mg$^{2+}$ (and the related Ca$^{2+}$) is consistent with the presence of Mg$^{2+}$ ion in the active site of the enzyme\cite{80,16}. 

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Figure 3.7: Effect of various heavy metals on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and 30 mM of HgCl₂, CuCl₂, CdCl₂, CaCl₂, CoCl₂, MgCl₂, or NaCl) at room temperature. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean of triplicates, except for MgCl₂ and NaCl data which are from single determinations.
3.4 Effect of calcium and mercury together on ALP activity.

Since Hg$^{2+}$ shown a very strong inhibitory effect on ALP and Ca$^{2+}$ showed just the opposite, we wondered if Ca$^{2+}$ could be used to alleviate the negative effect of Hg$^{2+}$ and ALP.

Table 3.6 and fig 3.8 showed Ca$^{2+}$ in the presence of Hg$^{2+}$ was not able to confer any protection to ALP, as Ca$^{2+}$/Hg$^{2+}$ together showed almost the same profile as Hg$^{2+}$ alone. This is an interesting finding and seems to suggest that the binding site of Hg$^{2+}$ is different than that of Ca$^{2+}$. Perhaps, one could speculate that Hg$^{2+}$ is binding to the Zn$^{2+}$ binding site, whereas Ca$^{2+}$ is going to the Mg$^{2+}$ binding site.
Figure 3.8: Effect of calcium and mercury together on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CaCl₂ and HgCl₂ at room temperature. The two heavy metals were added in equal concentrations to give the indicated concentration of total bivalent metal ions in the reaction mixture. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.6: Effect of calcium and mercury together on ALP activity

<table>
<thead>
<tr>
<th>$[\text{Ca}^{2+}]$ and $[\text{Hg}^{2+}]$, mM</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>49.7</td>
<td>8.1</td>
</tr>
<tr>
<td>10</td>
<td>29.9</td>
<td>5.2</td>
</tr>
<tr>
<td>20</td>
<td>6.8</td>
<td>1.4</td>
</tr>
<tr>
<td>30</td>
<td>1.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>
3.5 Effect of calcium and cadmium together on ALP activity

In contrast to what was observed for Ca$^{2+}$ and Hg$^{2+}$, in the co-incubation study we found that incubation of Ca$^{2+}$ significantly reduces the inhibitory effect of Cd$^{2+}$ when they were used together (table 3.7, fig 3.9).

This is also an interesting observation and suggests that Cd$^{2+}$ and Ca$^{2+}$ may be competing for binding to the same site. This finding also suggests that perhaps Ca$^{2+}$ could be potentially used to treat Cd$^{2+}$ contaminated soil.
Figure 3.9: Effect of calcium and cadmium together on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CaCl₂ and CdCl₂ at room temperature. The two heavy metals were added in equal concentrations to give the indicated concentration of total bivalent metal ions in the reaction mixture. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.7: Effect of calcium and cadmium together on ALP activity

|Ca^{2+}| and |Cd^{2+}|, mM | Normalized Rate | SD  |
|---|---|---|---|---|
|0  | 100.0 | 6.9 |
|5  | 96.7 | 6.0 |
|10 | 82.4 | 11.9|
|20 | 69.7 | 7.8 |
|30 | 67.0 | 7.0 |
3.6 Effect of copper and mercury together on ALP Activity

We were also interested in seeing how ALP would respond to co-incubation of two inhibitory heavy metals together.

Fig 3.9 and table 3.10 show that, these two heavy metals together had much more of an inhibitory effect than alone. This finding is very significant and further highlights the cumulative nature of the dangers of heavy metals pollution and especially on the activity of important enzymes.
Figure 3.10: Effect of copper and mercury together on ALP activity. As described under Materials and Methods, 4.5 nM ALP was added to a reaction mixture containing 33.33 µg/ml pNPP in 50 mM Tris, pH 8.5, and increasing amounts of CuCl₂ and HgCl₂ at room temperature. The two heavy metals were added in equal concentrations to give the indicated concentration of total bivalent metal ions in the reaction mixture. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.8: Effect of copper and mercury together on ALP activity

<table>
<thead>
<tr>
<th>[Cu$^{2+}$] and [Hg$^{2+}$], mM</th>
<th>Normalized Rate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>21.6</td>
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<tr>
<td>10</td>
<td>1.8</td>
<td>0.2</td>
</tr>
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<td>20</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>30</td>
<td>0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>
3.7 Effect of heavy metals and UV together on ALP activity

3.7.1 Effect of copper and UV together on ALP activity

Since many of heavy metals are known to produce oxy-radicals (and hence oxidative stress) in vivo, we wanted to test how exposure to UV radiation (a potential source of oxy-radicals) in the presence or absence of heavy metals would affect ALP.

As can be seen in figure 3.11, and table 3.9, UV exposure to ALP did not affect activity of the enzyme. However, when used together with Cu\(^{2+}\), UV radiation appears to further exaggerate the inhibitory effect of Cu\(^{2+}\) and cause increased inhibition of ALP. This is consistent with various reports that Cu together with UV radiation is much more toxic than either UV or Cu alone \(\text{Li and Trush, 1993}\)\(^{81}\) and \(\text{Kim et al 2009}\)\(^{82}\). The toxic effects of Cu\(^{2+}\) are attributed to its ability to generate oxyradicals through autoxidation reactions, as shown below (\text{Li and Trush, 1993 and Schützendübel and Polle 2002}\)\(^{83}\)

\[
\text{UV} \\
\text{Cu}^{+} + O_2 \rightarrow \text{Cu}^{2+} + O^{2-}.
\]

\[
O^{2-} + H_2O \rightarrow H_2O_2 + OH^{-}
\]

The \(H_2O_2\) produced can further undergo "Fenton-like" reactions to produce the very reactive hydroxyl radicals as well, as shown below (\text{Alnuaimi et al, 2007}\)\(^{84}\)

\[
H_2O_2 + Cu^{+} \rightarrow \text{OH}^+ + \text{OH}^- + \text{Cu}^{2+}
\]
Hence, copper (or other redox-cycling heavy metals) together with UV radiation can eventually lead to the production of reactive oxyradicals which can easily damage soil enzymes and microbes.

Since almost all soil is exposed to UV radiation this finding suggests that copper-contaminated soil could potentially cause very serious oxyradical-induced damages to ALP and other microbial enzymes.
Figure 3.11: Effect of copper and UV together on ALP activity. As described under Materials and Methods, ALP was exposed to 1mM CuCl₂, UV radiation (254 nm at a distance of 10 cm) or UV radiation together with 1mM CuCl₂ for the indicated times and then the “treated ALP” was tested for activity. The final concentration of “treated ALP” was 4.5 nM ALP, and the reaction mixture contained 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.9: Effect of copper and UV together on ALP activity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cu$^{+2}$ alone</th>
<th>UV alone</th>
<th>Cu$^{+2}$ &amp; UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
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<td>3</td>
<td>100</td>
</tr>
<tr>
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<td>74.4</td>
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<td>103.4</td>
</tr>
<tr>
<td>10</td>
<td>43.4</td>
<td>1.5</td>
<td>102.7</td>
</tr>
<tr>
<td>15</td>
<td>36.1</td>
<td>0.03</td>
<td>97.3</td>
</tr>
</tbody>
</table>
3.7.2 Effect of mercury and UV together on ALP activity

Examination of the effect of UV and Hg$^{2+}$ on ALP activity showed that unlike the Cu$^{2+}$ effect observed previously, UV exposure with Hg$^{2+}$ did not cause any additional inhibition of ALP. In fact, as shown in figure 3.12, and table 3.10, it appears that UV somehow decreases the inhibitory effect of Hg$^{2+}$. This is not surprising as UV radiation only works with redox cycling heavy metals to produce oxy-radicals.$^{81}$

Although, we are unable to explain the small “protective role” of UV radiation when used in the presence of Hg$^{2+}$, one could perhaps hypothesize that UV photolysis of water could generate small amounts of hydroxyl radicals and hydroxide anion which somehow could react with Hg$^{2+}$ to decrease its concentration in the solution.
Figure 3.12: Effect of mercury and UV together on ALP activity. As described under Materials and Methods, ALP was exposed to 1 mM HgCl₂, UV radiation (254 nm at a distance of 10 cm) or UV radiation together with 1 mM HgCl₂ for the indicated times and then the “treated ALP” was tested for activity. The final concentration of “treated ALP” was 4.5 nM ALP, and the reaction mixture contained 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.10: Effect of mercury and UV together on ALP activity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>UV alone</th>
<th>Hg$^{+2}$ + UV</th>
<th>Hg$^{+2}$ alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
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<td>99.2</td>
<td>7.3</td>
<td>74.0</td>
</tr>
<tr>
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<td>103.4</td>
<td>7.2</td>
<td>45.7</td>
</tr>
<tr>
<td>10</td>
<td>102.7</td>
<td>8.1</td>
<td>20.3</td>
</tr>
<tr>
<td>15</td>
<td>97.3</td>
<td>8.7</td>
<td>13.9</td>
</tr>
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</table>
3.7.3 Effect of cadmium and UV together on ALP activity

Similar to what observed for Hg\(^{2+}\), UV exposure in the presence of Cd\(^{2+}\) did not lead to any extra inhibition of ALP (figure 3.13 and table 3.11).

Again (like Hg\(^{2+}\)), Cd\(^{2+}\) is not a redox-cycling heavy metal (as the case with Cu\(^{2+}\)) and therefore should not lead to generation of oxyradicals when exposed to UV radiation.
Figure 3.13: Effect of cadmium and UV together on ALP activity. As described under Materials and Methods, ALP was exposed to 1 mM CdCl₂, UV radiation (254 nm at a distance of 10 cm) or UV radiation together with 1 mM CdCl₂ for the indicated times and then the "treated ALP" was tested for activity. The final concentration of "treated ALP" was 4.5 nM ALP, and the reaction mixture contained 33.33 µg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.11: Effect of cadmium and UV together on ALP activity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cd(^{12}) alone Mean</th>
<th>Cd(^{12}) alone SD</th>
<th>UV alone Mean</th>
<th>UV alone SD</th>
<th>Cd(^{12})+UV Mean</th>
<th>Cd(^{12})+UV SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>89.0</td>
<td>1.7</td>
<td>99.2</td>
<td>7.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>77.9</td>
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<td>103.4</td>
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<td>82.7</td>
<td>8.7</td>
</tr>
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<td>82.7</td>
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<td>97.3</td>
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<td>90</td>
<td>12.06</td>
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</table>
3.8 Effect of H$_2$O$_2$ on the ALP activity

We wanted to further study the effect of oxy radicals on ALP and so we next looked at the effect of H$_2$O$_2$ in presence of various heavy metals.

As can be seen in figure 3.14, incubation of H$_2$O$_2$ with ALP led to a time–dependant decrease in ALP activity. This is most likely due to direct effect of the strong oxidant H$_2$O$_2$ reacting with ALP and oxidizing (damaging) the protein, as has been documented extensively in scientific literature.$^{[85]}$
Figure 3.14: Effect of H$_2$O$_2$ on the ALP activity. As described under Materials and Methods, ALP was incubated with 0.25 mM H$_2$O$_2$ for the indicated times and then the "treated ALP" was tested for activity. The final concentration of "treated ALP" was 4.5 nM ALP, and the reaction mixture contained 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any hydrogen peroxide (control). Data shown is the mean and standard deviation of triplicates.
3.8.1 Effect of mercury and hydrogen peroxide together on ALP activity

Since both Hg\(^{2+}\) and H\(_2\)O\(_2\) when used alone inhibited ALP activity, it was expected that when used together, the inhibitory effect would be much greater. As expected and as shown in figure 3.15, and table 3.12, incubation of H\(_2\)O\(_2\) with Hg\(^{2+}\) further increased the inhibitory effect of Hg\(^{2+}\) on ALP.

It is well known that many heavy metals can react with H\(_2\)O\(_2\) to produce even more damaging reactive oxygen species \(^{186}\). Perhaps, the same sorts of reactions are happening here, which are producing oxyradicals and eventually damaging ALP.
Figure 3.15: Effect of mercury and hydrogen peroxide together on ALP activity. As described under Materials and Methods, ALP was exposed to 1 mM HgCl₂, 0.25 mM H₂O₂, or 0.25 mM H₂O₂ with 1 mM HgCl₂ for the indicated times and then the "treated ALP" was tested for activity. The final concentration of "treated ALP" was 4.5 nM ALP, and the reaction mixture contained 33.33 µg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.12: Effect of mercury and hydrogen peroxide together on ALP activity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Hg(^{2+}) alone Mean</th>
<th>SD</th>
<th>H(_2)O(_2) alone Mean</th>
<th>SD</th>
<th>Hg(^{2+}) + H(_2)O(_2) Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>82.6</td>
<td>10.1</td>
<td>43.1</td>
<td>4.3</td>
</tr>
<tr>
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<td>57.1</td>
<td>0</td>
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<td>3.3</td>
<td>22.6</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>27.2</td>
<td>10.2</td>
<td>48.0</td>
<td>6.1</td>
<td>14.9</td>
<td>0.4</td>
</tr>
<tr>
<td>20</td>
<td>16.2</td>
<td>2.6</td>
<td>42.1</td>
<td>5.1</td>
<td>11.0</td>
<td>0.7</td>
</tr>
<tr>
<td>30</td>
<td>11.5</td>
<td>1.2</td>
<td>37.9</td>
<td>4.7</td>
<td>7.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>
3.8.2 Effect of the cadmium and hydrogen peroxide together on ALP activity

Similarly as shown in figure 3.16 and table 3.13, the activity of the enzyme was strongly inhibited by applying H\textsubscript{2}O\textsubscript{2} in presence of Cadmium more than the inhibition by H\textsubscript{2}O\textsubscript{2} or Cd\textsuperscript{2+} alone.

However, the effect of H\textsubscript{2}O\textsubscript{2} when used together with Cd\textsuperscript{2+} was much more dramatic than seen with Hg\textsuperscript{2+}.

In the case of Hg\textsuperscript{2+}, the increased in inhibition caused by H\textsubscript{2}O\textsubscript{2} appeared to be only marginal (5-20%), when compared to Hg\textsuperscript{2+} alone. However, in the case of Cd\textsuperscript{2+}, the H\textsubscript{2}O\textsubscript{2} effect appears to be synergistic (and not just additive). This is a very interesting observation which needs further investigation at a later time. However, the data seems to imply that perhaps Cd\textsuperscript{2+} is much more reactive than Hg\textsuperscript{2+} in reacting with H\textsubscript{2}O\textsubscript{2} and generating oxy-radicals. Alternatively, it can damage ALP by replacing the Zn from the active site as has been suggested by Poirier et al. 2008[721] Lastly, Cd\textsuperscript{2+} could also be reacting directly with the sulphhydryl groups on ALP and damaging the enzyme [87]. Hence, Cd\textsuperscript{2+} in the presence of H\textsubscript{2}O\textsubscript{2} could be damaging ALP by any combination of the above mentioned 3 mechanisms.
Figure 3.16: Effect of the cadmium and hydrogen peroxide together on ALP activity.

As described under Materials and Methods, ALP was exposed to 1 mM CdCl₂, 0.25 mM H₂O₂, or 0.25 mM H₂O₂ with 1 mM CdCl₂ for the indicated times and then the “treated ALP” was tested for activity. The final concentration of “treated ALP” was 4.5 nM ALP, and the reaction mixture contained 33.33 μg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.13: Effect of cadmium and hydrogen peroxide together on ALP activity

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cd\textsuperscript{2+} alone Mean</th>
<th>SD</th>
<th>H\textsubscript{2}O\textsubscript{2} alone Mean</th>
<th>SD</th>
<th>Cd\textsuperscript{2+}+H\textsubscript{2}O\textsubscript{2} Mean</th>
<th>SD</th>
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</table>
3.8.3 Effect of copper and hydrogen peroxide together on the ALP activity

In contrast to what we saw with \( \text{Hg}^{2+} \) and \( \text{Cd}^{2+} \), incubation of \( \text{H}_2\text{O}_2 \) with \( \text{Cu}^{2+} \) produced very unexpected result.

As can be seen from figure 3.16, and table 3.14, presence of \( \text{H}_2\text{O}_2 \) appeared to decrease the inhibitory effect of \( \text{Cu}^{2+} \) on ALP activity. We are unable to explain this strange (but reproducible) observation. It is possible that \( \text{H}_2\text{O}_2 \) is somehow reacting with \( \text{Cu}^{2+} \) to form an inactive complex which is not capable of interacting with ALP.
**Figure 3.17**: Effect of copper and hydrogen peroxide together on ALP activity. As described under Materials and Methods, ALP was exposed to 1 mM CuCl₂, 0.25 mM H₂O₂, or 0.25 mM H₂O₂ with 1 mM CuCl₂ for the indicated times and then the “treated ALP” was tested for activity. The final concentration of “treated ALP” was 4.5 nM ALP, and the reaction mixture contained 33.33 µg/ml pNPP in 50 mM Tris, pH 8.5. The rate of ALP catalysis was calculated from the linear portion of the absorbance (405 nm) vs time graph and was normalized to the rate obtained without any heavy metal (control). Data shown is the mean and standard deviation of triplicates.
Table 3.14: Effect of copper and hydrogen peroxide together on ALP activity

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<th>Time (min)</th>
<th>( \text{Cu}^{2+} ) alone Mean</th>
<th>( \text{Cu}^{2+} ) alone SD</th>
<th>( \text{H}_2\text{O}_2 ) alone Mean</th>
<th>( \text{H}_2\text{O}_2 ) alone SD</th>
<th>( \text{Cu}^{2+}+\text{H}_2\text{O}_2 ) Mean</th>
<th>( \text{Cu}^{2+}+\text{H}_2\text{O}_2 ) SD</th>
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</tr>
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</table>
Chapter IV

CONCLUSION
Conclusion

In conclusion, this study reports on the effect of various heavy metals, UV radiation, and Hydrogen peroxide, in alone or in combination on E. coli ALP.

Our results showed that of the heavy metals tested Hg^{2+}, Cu^{2+}, and Cd^{2+} significantly inhibited the enzyme. This is similar to what others have seen with whole soil samples as well as immobilized ALP enzymes. However, in our assay system, it seems that we had to use much higher concentrations of heavy metals to see an inhibition. This could be due to differences in the enzyme preparation we are using (from Sorachim, France), or perhaps E. coli ALP is a little bit different than the ones reported by other investigators. Cobalt on the other hand did not cause inhibition of the enzyme, rather incubation of 5 mM Co^{2+} with ALP appears to activate the enzyme. This activation of ALP by Co^{2+} has also been observed previously for ALP from other organisms, however, it is noteworthy that there is at least one report which shows that Co^{2+} can actually inhibit ALP.

As expected, since ALP is a Zn-Mg metallo-enzyme, with Zn^{2+} ions and Mg^{2+} ion in it's active site, incubation of divalent metal ions like Ca^{2+} and Mg^{2+} activated the enzyme. Therefore, the inhibitory effects of Hg^{2+}, Cu^{2+} and Cd^{2+} observed here were not just because they are divalent metal ions. Co-incubation experiments with Ca^{2+} and Cd^{2+} together show that Ca^{2+} can significantly decrease the toxicity of Cd^{2+}. This is an interesting observation and points to a possible use of Ca^{2+} or Mg^{2+} as a remediation for Cd^{2+}-contaminated soil.

UV radiation by itself did not have much of an effect on ALP activity. However, together with some of the heavy metals the combination resulted in significant decrease in ALP activity. We believe this to be due to the possible production of oxy-radicals where heavy metals are exposed to UV radiation.
Lastly, incubation of H₂O₂ caused a concentration and time dependent decrease in ALP activity. We found that inhibitory effect of heavy metals could be either further increased (e.g. Hg²⁺, Cd²⁺) or decreased (e.g Cu²⁺) by the incubation with H₂O₂.

In summary this study shows that heavy metal pollution (together with UV radiation and or oxidants) can dramatically affect the activity of a very important nutrient-recycling enzyme in the soil namely ALP. This study underscores the ease with which soil (and enzymes present in it) can be damaged by various environmental pollutants.
References


البنفسجية والزئبق يثبط عمل الإنزيمات أكثر من تأثير المعاد التقليلة بمفردها. وعلاوة على ذلك فأن فوق اكسيد الهيدروجين سواء كان بمفرده أو عند إتجاه مع المعاد التقليلة فإنه يعمل على تثبيط نشاط الإنزيم الفوسفتيز القلوي على حد سواء.

ولقد أوضح هذه البيانات إن الإنزيم الفوسفتيز القلوي البكتيري شديد الحساسية للمعاد التقليلة وتؤدي إلى تثبيته وكذلك الظروف التي تساعده في زيادة الإنتاجة الإيجابية الحرارة ومدى تعطيلها للأوزيم باستخدام المعادن التقليلة المختلفة. باختصار، تبين لنا أن التلوث بالمعادن التقليلة يثير قلقاً بالغاً على حالة ميكروبات التربة، الذي لديها القدرة على التأثير في الزراعة والنتاج الغذائي بشكل مباشر.
10 - يمكن أن ترتبط المعادن القليلة بالعديد والكائنات الداخلية لكل من (SOD) أو (GSH) أو (GSH) أو (SOD) إلى تثبيط نشاطها.

3. هذه المعادن القليلة يمكنها أيضًا أن تتفاعل في ارتباطها بالإنزيمات والتي تؤدي إلى تثبيط نشاطها.

لقد أثبتت الدراسات الحديثة أن تلوث التربة بالمغذيات القليلة لا يؤثر فقط على الكائنات الدقيقة بل يؤثر على نشاط الإنزيمات التي تفرزها مثل الديهيدرو جينات، بيتا جيلكو سيدان، السيلوز، تيبول، الأكسيداز، بير، اميان، سفينتاز، و الادسي لوير. ومعظم الاحصاءات تكون قائمة على مراقبة نشاط هذه الإنزيمات في التربة الملوحة بالمعادن القليلة. ولكن بما أن التربة تحتوي على مكونات كثيرة يمكن أن يكون لها تأثير على نشاط الإنزيمات وليس الارتباط المباشر بين المغذيات القليلة والإنزيمات. إضافة إلى أن الإنزيمات التي تتطلب الإنسمة قد تدخل في عملية الإيضاح للكائنات الحية الدقيقة أو تتأثر بالعناصر البيئية المختلفة مما يؤثر على نشاط الإنزيمات كتأثير ثانوي.

دراسات قليلة تم نشرها تخبر بالتأثير المخالفة للمغذيات القليلة على الإنزيمات الخارجية جسم الكائن الحي. لقد قمنا بالجذب تأثير المعادن القليلة المتاحة مثل ماغنيسيوم وكالسيوم وصوديوم والكالسيوم والمغليوبين ونظام الزيتي على إنزيم الفوسفات. ولقد أوضح.joda هذه الدراسة أن بعض المعادن القليلة مثل الزيتي والمغليوبين تعمل على تثبيط انزيم الفوسفات القلوي بقوة أكثر من الصوديوم والكالسيوم. وبالرغم من الفئات الأرضية ثنائية التكافؤ مثل الكالسيوم والمغليوبين تعمل على تثبيط الإنزيم والمغليوبين منشور قوي أكثر من الكالسيوم. أيضاً المركبات أحاديات التكافؤ مثل الصوديوم ليس لها تأثير على نشاط مركب الفوسفات. ولقد أبانا قمنا باختيار تأثير اتحاد المعادن القليلة على نشاط مركب الفوسفات مثل اتحاد بين كالسيوم مع كاديوم، واتحاد الكالسيوم مع الزيتي، واتحاد النحاس مع الزئبق.

أما في الجزء الثاني من الدراسة، لقد قمنا باختيار تأثير الارتفاع والتآكل مثل تأثير الأشعة فوق البنفسجية وفوق اليد وحين غربين على نشاط إنزيم الفوسفات. وقد قمنا أيضاً بتناول تأثير الأشعة فوق البنفسجية سوياً مع المعادن على نشاط إنزيم الفوسفات القلوي. ولقد لاحظنا ان الأشعة فوق البنفسجية بنفسها ليس لها تأثير على الإنزيم. وبالرغم من ذلك فإن المركب بين الأشعة فوق البنفسجية والنحاس، كذلك الأشعة فوق...
الملخص

تعتبر التربة مصدرًا من المصادر الطبيعية ذات أهمية عظيمة للزراعة والبيئة على سواء. إن التربة الجيدة صحية لا تساعد فقط في تحسين جودة المنتجات الزراعية فقط بل إنها مهمة أيضًا في إعادة تدوير مكونات التربة العضوية ومع ذلك فإن تلوث البيئة بالعديد من الملوثات مثل المعادن الثقيلة والمبيدات الحشرية أو المواد الهيدرو كربونية الناتجة من البترول قد تؤدي إلى مشاكل بيئية وحيوية خطيرة. إن هذا التلوث قد يضر مباشرة كل من الإنسان والحيوان كما وانه قد يفسد كل مصادر مياه الشرب والنباتات والخضروات إضافة إلى ذلك فإنه قد يؤثر على الحشرات والكائنات الحية الدقيقة التي تعيش في هذه التربة.

الكائنات الدقيقة التي تعتبر جزءا أساسيا من مكونات سطح التربة التي تلعب دورا حيويا في إعادة تدوير النيتروجين الفسفور والكربونين تماما مثل ما تقوم به المواد العضوية ولهدأ فالكيثيرا تؤثر على إعادة تدوير المواد الغذائية والكربون، والعناصر المختلفة الأخرى.

بالإضافة إلى إمكانية إعادة التدوير الغذائي، للكائنات التربة الدقيقة يمكنها أيضًا تأثر على خواص التربة الفيزيائية عن طريق إفراز مواد سكرية تعمل على تثبيت التربة، والتي تؤثر بدورها على الاحتفاظ بالماء ومعدل التربة تقشر وتصرح التربة وبالتالي فإن الصحة الجيولوجية تعتبر مؤشرًا جيدًا على صحة التربة.

إن تلوث التربة بالمعادن الثقيلة قد يظهر بواعث من الطرق الأربع المختلفة:

1 - أن المعادن الثقيلة يمكن أن تعيق عملية إعادة التدوير لأنها تؤثر على عمليات الأكسدة والاختزال تكريس الأيونات الحررة والتي تتسبب اختلال في خلايا الكائنات الحية.

2 - عن طريق ارتباط المعادن الثقيلة بالمجموعة الكربونية الموجودة في البروتينات مما يؤدي إلى تغيير تركيزها والتأثير على شكلها.
جامعة الإمارات العربية المتحدة
عمادة الدراسات العليا
برنامج ماجستير علوم البيئة

تأثير الملوثات المختلفة على نشاط إنزيم الفوسفتيز القلوي البكتيري

رسالة مقدمة من الطالبة

ميثة مكتوم سالمين التعيمي

مقدمة إلى/

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استكمالاً لمعايير الحصول على درجة الماجستير في علوم البيئة

2010 - 2009
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برنامج ماجستير علوم البيئة

تأثير الملوثات المختلفة على نشاط إنزيم الفوسفيتاز القلوي البكتيري

رسالة مقدمة من الطالبة

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2010 - 2009

Shrien