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# Effect of Pig Iron Slag Particles on Soil Microbial and Enzyme Activities

[1]Ediga Anjaneyulu, [1]Mopuri Ramgopal,[2]Golla Narasimha and [1]Meriga Balaji

[1]Department of Biochemistry, Sri Venkateswara University, Tirupati-517502, India
[2]Departments of Virology, Sri Venkateswara University, Tirupati-517502, India

**Abstract:** The effect of pig iron slag particles on soil physico-chemical, biological and certain soil enzyme properties was studied. Contamination of iron slag particles altered physico-chemical, biological and enzyme properties of soil. While soil pH increased slightly, electrical conductivity, carbon, potassium and phosphorus contents increased significantly in polluted soil. Contamination of soil with pig iron slag caused drastic reduction in microbial population. Similarly, enzyme activities such as dehydrogenase and protease were ceased by three fold in polluted soil when compared to the control.

**Key words:** Pig iron slag · Physico-chemical · Biological properties · Soil enzymes

## INTRODUCTION

Soil is a dynamic system in which continuous interaction takes place between soil minerals, organic matter and organisms. Each of these three major soil components influences the physico-chemical and biological properties of terrestrial system [1]. Metals are natural constituents of the earth's crust but anthropogenic activities have resulted in drastic alterations in their geochemical cycles causing pollution and biochemical imbalance in the biosphere. In recent decades the development of industry and agriculture and activities such as mining, smelting of metal ions, industrial emissions and application of agrochemicals and fertilizers contributed to elevated levels of metals including iron in soils of Rayalasema region of Andhra Pradesh, India. Although iron is required in trace amounts for living organisms, discharge and deposition of iron ore based industrial contaminants have become an environmental issue of great public concern owing to their adverse affects on soil flora and fauna and ultimately to mankind.

Since soil enzyme activities are very sensitive to pollution, enzymes have been suggested by some researchers as potential indicators or monitoring tools to assess soil quality [2-3]. Bioremediation activities [4]. Numerous changes with respect to physico-chemical, biological and enzymatic parameters were observed in soils contaminated with effluents discharged from cotton ginning mill [5]. Alcohol and chemical industries [6]. Pulp and paper industry [7]. Enzyme activities that influence functional processes occurring in a given soil play an important role in determination of soil profile and biological activity [8]. Pollution of the soil environment with heavy metals, their effect on the soil microorganisms and on their enzymatic activity depend, apart from other things, on the soil pH, the content of organic and mineral colloids and on a metal type and its chemical properties[9].

There is a considerable interest in the study of soil physico-chemical properties and enzyme activities which may reflect the potential of a soil to form certain biological transformations related to soil fertility [10]. In view of the increasing environmental pollution, decreasing soil fertility and adverse effects on soil flora and fauna, it is of utmost concern to study the effects of metal pollutants on soil characters and soil microorganisms which pave the way for greater understanding in the direction of improving soil fertility or bioremediation. Since iron ore is one of the most extensively mined, smelted, processed and used metal for majority of purposes, iron ore based industrial discharge and contaminants have become a major issue of environmental concern. An attempt was made in the present study with an aim to determine the effect of iron ore slag of pig iron plant on soil physic-chemical, biological and enzyme activities of dehydrogenase, protease, cellulase and amylase.

**Corresponding Author:** Meriga Balaji, Department of Biochemistry, Sri Venkateswara University, Tirupati-517502, India, E-mail: balaji.meriga@gmail.com.

## MATERIALS AND METHODS

**Soil Collection:** Soil samples were collected from dumpings of pig iron plant located at Srikalahasti, Chittoor district of Andhra Pradesh, India and this was treated as test soil and the control was collected away from the pig iron pant. Prior to testing, the soils were air-dried, passed through a 2 mm sieve and stored at room temperature for further studies.

**Physico-Chemical Properties of Soil:** The percentage of coarse fragments was quantified from the weight of material retained after sieving through a 2 mm sieve [11]. Soil pH and electrical conductivity were determined by pH meter (Elico) and conductivity meter respectively. Water holding capacity and organic carbon content were quantified by the method of Johnson and Ulrich [12]. Walkely and Black [13]. Soil phosphorus and potassium contents were determined respectively by the methods of Watanabe and Olsen [14]. Toth [15].

**Enumeration of Soil Microorganisms:** Soil microbial population including bacterial and fungal populations were enumerated by serial dilution technique. One gram of each soil sample was serially diluted and 0.1 ml was spreaded with a sterile spreader on nutrient agar media (pH 7.2) and potato dextrose agar medium for the growth of bacteria and fungi respectively. After incubation period, colonies formed on the surface of the medium were counted by colony counter.

**Assay of Dehydrogenase:** The dehydrogenase activity was determined by the modified procedure of Casida [16]. To five grams of soil in a test tube, 2.5 ml sterile distilled water and 1ml 3% aqueous solution of triphenyl tetrazolium chloride (TTC) was added. These tubes were mixed thoroughly and incubated at 30°C for 24 h. The liberated end product, triphenyl tetrazolium formazone was measured at 485nm in spectrophotometer (Elico).

**Assay of Protease:** Five grams of soil sample was placed in 50 ml Erlenmeyer flasks and 6 ml of 0.2M acetate buffer (pH 5.5) containing 1% BSA was added and incubated for 6 h at 30°C. After incubation, 1ml of toluene was added and the suspension filtered through Whattman No.1 filter paper. The amount of protein content in the filtrate was determined by Folin-Lowry method [17].

## RESULTS

**Physico-chemical Properties of Soil:** The soil polluted with iron slag increased soil pH from 7.2 to 7.9, water holding capacity 0.3 to 0.7 ml/g of soil and electrical conductivity 0.6 to 1.13 Mmhos/cm Similarly higher organic carbon, phosphorus and potassium levels were observed in test soil (Table 1).

**Soil Microorganisms:** Polluted soil caused 2-3 fold decrease in bacterial and fungal population compared to control soil. For instance, the bacterial and fungal population in control and polluted soil was $142x10^5$, $86x10^5$ cfu/g and $25x10^5$ and $7x10^5$ cfu /gram of soil respectively.

**Soil Dehydrogenase Activity:** The dehydrogenase activity of polluted soil was drastically inhibited (8-9 fold) when compared to control soil. With increase in soil incubation period, dehydrogenase activity also increased up to $16^{th}$ day and decreased beyond that (Fig. 1).

**Soil Protease Activity:** Like dehydrogenase, protease activity was also inhibited (3-4 fold) in polluted soil. With increase in soil incubation period, protease activity also increased up to$16^{th}$ day incubation but decreased thereafter (Fig. 2).

Table 1: Physico-chemical properties of soil contaminated with/without slag particles discharged from pig iron plant.

| Physico chemical properties | Control soil | Test soil |
|---|---|---|
| Colour | Light Brown | Black |
| Odor | Normal | Unpleasant |
| Soil nature | High weight | Low Weight |
| pH | 7.2 | 7.9 |
| Electrical onductivity (Mmhos/cm) | 0.6 | 1.13 |
| Water holding capacity (ml/g of soil) | 0.3 | 0.7 |
| Organic carbon (%) | Low | Medium |
| Availability of phosphorus Kg/ha | 21 | 30 |
| Availability of potassium Kg/ha | 73 | 130 |

Table 2: Microbial population (*CFU) in soil contaminated with/without slag particles discharged from pig iron plant.

| Microorganisms | Control soil | Test soil |
|---|---|---|
| Bacteria | $142x10^5$ | $86x10^5$ |
| Fungi | $25x10^5$ | $7x10^5$ |

*Microbial populations measured in terms of colony forming units (CFU/g) of soil

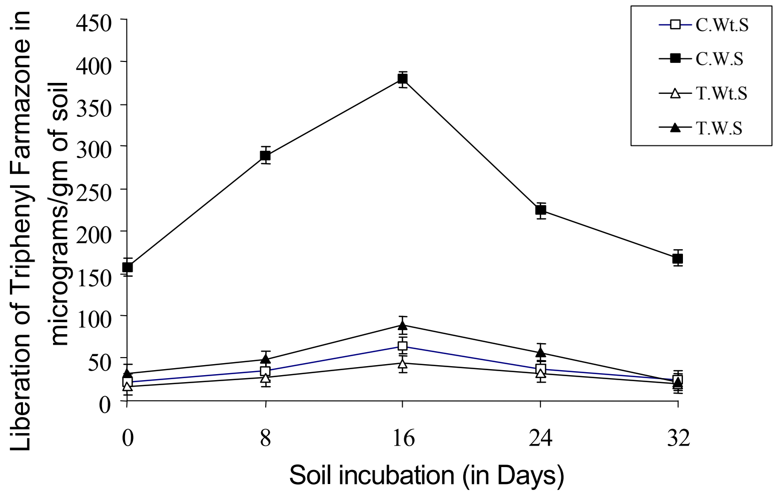


Fig. 1: Dehydrogenase activity of soil contaminated with/without slag particles discharged from pig iron plant. (Activity in terms of release of µg of Triphenyl formazone / gm of soil)
C.Wt.S: Control with out substrate; C.W.S: Control with substrate;
T.Wt.S: Test with out substrate; T.W.S: Test with substrate.

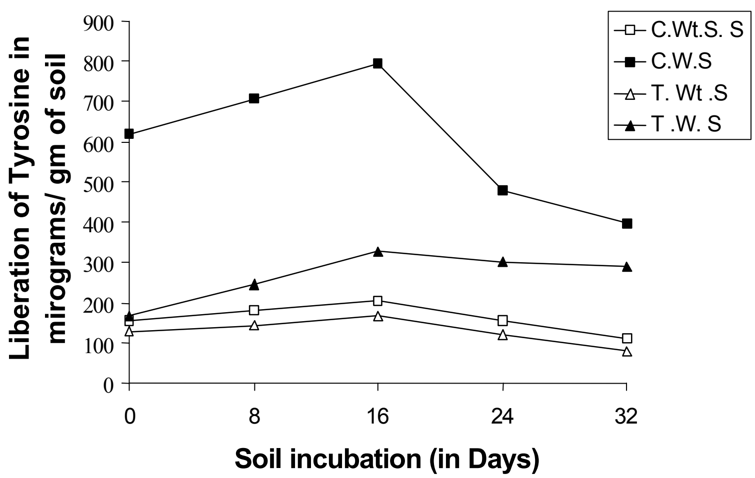


Fig. 2: Protease activity of soil contaminated with/without slag particles discharged from pig iron plant. (Activity in terms of release of µg of tyrosine / gm of soil)
C.Wt.S: Control with out substrate; C.W.S: Control with substrate;
T.Wt.S: Test with out substrate; T.W.S: Test with substrate.

## DISCUSSION

Our observations revealed that contamination with iron slag particles led to increase in soil pH of the test soil (Table 1). This indicates the alkalin nature of the disposed maste contaminants. Increase in soil pH was also reported by aluminium and heavy metal contaminated soils [18]. Increased water holding capacity and electrical conductivity observed in test soil reflects the altered chemical and biological characters of the polluted soil. Similar reports were observed in long term discharge of sewage effluents [19]. Cotton ginning mill effluents [5]. In contrast, soils polluted with cement dust from cement industries had low water holding capacity and higher electrical conductivity [18-20]. The higher organic matter, phosphorus and potassium observed in polluted soil (Table 1) may be due to the effect of discharged effluents from pig iron plant. Similar results were noted in soils contaminated with pesticide industrial effluents [21]. long term municipal waste disposed soils [22]. Discharge of effluents from cotton ginning mills [5]. Heavy metals content in vetiver grown on iron ore tailings [23].

Reduced bacterial and fungal population in polluted soil may be due to the toxic effects of iron slag particles on microbial population. The oligotrophic microbes showed the highest sensitivity to iron and certain heavy metal pollution, indicating that the limitation of microbial community is more pronounced in soils poor in organic matter and nutrient content [24]. Pollution of the soil environment with heavy metals also negatively influenced the soil microbial properties such as basal soil respiration rate and enzyme activities depending on the soil pH, organic matter content and other chemical properties [25-4].

Enzymes are strongly connected with important soil characteristics such as organic matter, physical properties, microbial activity or biomass. They are the sensitive indicators of soil quality.

In the present study, very significant inhibition of dehydrogenase and protease activities in polluted soil indicates the alterations in oxidation-reduction activities of enzymes released from microorganisms(fig-1&2). It is considered that iron also like other heavy metals, mainly inhibit enzymatic reactions through either their complexing with substrate or blocking the functional groups of enzymes or reacting with complex enzyme-substrate. Similar results were observed in dehydrogenases activity in flooded soils [10]. in herbicides contaminated soils [26]. Reduced protease activity was observed in soils contaminated with insecticides [27]. Chlorothionil [28]. In contrast, soil treated with tomato processing waste [29], cotton ginning mill [30] and dairy shed effluents [31]. Resulted in increase in soil protease activity than the control.

## CONCLUSION

The present study clearly indicates that dumping of pig iron slag on soil, altered the physico-chemical, biological properties and inhibited soil dehydrogenase and protease activities in the soil.

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