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Serum protein levels of *Channa striata* (Murrel) exposed to coal dust /ash of Godavari Khani (Telangana)

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Abstract

Coal dust and coal ash are coal pollutants emanating from Godavari Khani, of Telangana. These pollutants, which are effluents formed as result of extensive mining of coal fields, of Ramagundam, thermal power plant, have greatly impacted the local environment. The waters of the local river, Godavari and the surrounding areas have been consistently reporting degradation of air and water quality. Quite a number of studies on coal pollution, have reported that coal contaminated air and water has a deep impact upon human health apart from causing deterioration of surrounding flora and fauna. Though this has been a age old problem, of this area, not many systematic studies, have been conducted, to explain or reveal the physiological impacts about coal pollution. The present study was undertaken to study the serum protein levels, serum albumin and serum globulin in the blood *of Channa striata*, (murrel) a common fish from local waters. Fish were captured and maintained under laboratory conditions, and were exposed to different concentrations of coal dust and coal ash (0.5%, 1%, 2%).after the experimental period of seven days, the blood was collected from the different groups of fish and serum proteins, serum albumin were estimated and calculated. Our results, showed a significant decline in the protein levels when compared to that of the control fish. Analysis of variance was done (ANOVA) to know the level of significance. Our results were significant at $P< 0.01$. This study clearly demonstrates that coal ash and coal dust even in small concentrations, greatly impact the protein levels in the blood suggesting liver damage.

Godavarikhani, which means is a collection of mines, namely GDK 1&1A, GDK 2&2A and GDK5 have a capacity of 1.54 MTPA on a ML area of 1272.44 Ha and are located in the Mandal Ramagundam and District Karimnagar in the state of Telangana, India. Godavarikhani is also well known as coal city, in the neighborhood of Ramagundam in the district of Peddapalli, Telangana state. Godavari Khani is situated on the banks of the river Godavari. Heavy mining activity in and around this river, has lead to heavy pollution

due coal dust and coal ash. Apart from coal pollution, in this area, Radon pollution in the air of coal mines of Godavari khani has been reported by Rao *et.al,*[8]. Reports in local daily[6] reported that, the coal ash waste from the coal mines, is frequently dumped in the Ramagundam ponds, polluting the waters, causing gastrointestinal diseases in humans and also drastically affecting the aquatic life. The long-term effects of coal mining lead to a gradual disintegration of green environment in around the fields and also seems to have a pessimistic impact of the water bodies too and also the quality of soil is also drastically affected. *et.al*.,[3]. The main factor for heavy pollution is in the water bodies near the thermal plants may be attributed to indiscriminate disposal of coal waste in to the waters as suggested by Singh,[11]. The particulate pollution caused by coal dust and ash, seems to alter the physical and chemical properties of water[9] and a drastic affect upon aquatic life can be seen is mostly because of alternation of pH which may been caused due to untreated disposal of water waste[13]. Authors like Siboni, Fine, Bresler, & Loya,[12] suggest that the heavy metals which are find in the coal could be quite lethal and toxic to majority of the marine organisms. Studies by Hillaby[5], suggest that the increase in coal dust particles, over the gills, up to concentration of about 50 % of the body weight, can cause a reduction in the area of the gills, causing breathing difficulties leading to mortality in fish. From these studies it is quite evident that coal pollutants, have a great impact upon all kind of life systems, which includes, plant, animals and humans. Though the problem of coal pollution in Godavari khani is quite long in years, not many studies have been attempted to study its long term exposure upon biological life especially animal physiology. Therefore the present study, has been attempted to learn about the physiology of fish, (*Channa striata*) with reference to serum proteins under stress of exposure to coal dust and coal ash.

Channa striata, the common murrel was obtained, from local waters of Godavari river. After the catch, the fish were carefully transported to the laboratory. In the laboratory, fish were transferred in two large plastic tubs. They were acclimatized to laboratory conditions for 48 hours, before starting with the experimentation. They were fed *ad libitum* with commercially available fish feed (tubifex blocks) obtained from local aquarium. After acclimatization, fish were separated in to different groups. The experimental fish were treated, with different concentrations of coal ash and coal dust which were prepared using water as solvent. They were maintained in the laboratory for a period of 7 days, before the blood was drawn.

Group I: Control Fish

Group II : Treated fish (0.5% Coal Ash)

Group III : Treated fish (1% Coal Ash)

Group III : Treated fish (2% Coal Ash)

Group IV : Treated fish (0.5% Coal dust)

Group V : Treated fish (1% Coal Dust)

Group VI : Treated fish (2% Coal Dust)

Morphological characteristics of the fish are as follows :

Fish measuring approximately 30 cm in length, and weighing about 300-gms, were selected for the experimentation. The fish

were carefully taken out of the waters, and were dissected, to expose the heart tissue. The blood was drawn directly from the heart muscle using the syringe. the blood was immediately transferred in to the vials and was processed for experimentation.

Method :

The blood was withdrawn from the fish, and used for estimation of total serum proteins and also for albumin and also globulin was estimated. The following were estimated using the standard kits.

Estimation of proteins by Biuret Method:

Principle: Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet colored complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Reaction: Total Protein Cu Violet complex.

Contents: Reagent 1 : Biuret Reagent, **Reagent 2** : Protein Standard 6 g/dl

Materials Required - Clean & Dry Glassware. - Laboratory Glass Pipettes or Micropipettes & Tips. - Colorimeter or Bio-Chemistry Analyzer. Samples: Serum, heparinized/EDTA Plasma. Proteins are reported to be stable in the sample for 6 days at 2-8°C

Preparation of Reagent & Stability: All reagents are stable till the expiry date mentioned on the label at room temperature. Standard vial once opened should be stored at 2 -8°C, it is stable till the expiry date mentioned on the vial. All reagents are in ready to use form.

General system parameters: Reaction type : End point Wave length : 546 nm (530 - 570 nm) Temperature : Room temperature Incubation : 5 minutes Reagent volume : 1.0 ml Sample volume : 10 µl Standard concentration : 6 gm/dl. Zero setting : Reagent blank Light path : 1 cm

Procedure : Pipette into clean dry test tube labeled as Blank (B), Standard (S) and Test (T) : Addition sequence B. S. T. Biuret Reagent 1ml Standard - 10 µl - Sample - - 10 µl Mix well, Incubate for 5 minutes at Room temperature. Measure the absorbance of the standard Abs. S and sample Abs. T against the reagent blank , within 60 minutes.

Calculation : Total Protein Conc. (gm/dl)= Abs.

BCG (Bromocresol Green) Albumin Assay Kit for assay of serum albumin Catalog Number MAK124 :

The BCG (Bromocresol Green) albumin assay kit is designed to measure albumin directly without any pretreatment of samples, such as serum, plasma, urine, and biological preparations. The optimized formulation substantially reduces interference by other substances (lipids/other proteins) in the raw samples. Use ultrapure water for dilutions. Bring Reagent to room temperature and shake before use. Dilute Albumin Standard (5 g/dL) in ultrapure water. Transfer 20 mL of diluted standards, Blank, and diluted samples to appropriately labelled tubes. Add 1,000 mL

Serum Protein levels of *Channa striata* exposed to (Coal Dust)

1	Control	5.3 +_ 0.129
2	Coal Dust (0.5%)	4.3+_ 0.149
3	Coal Dust (1%)	4.1 +_ 0.170
4	Coal Dust (2%)	4.4 +- 0.091

Table-1

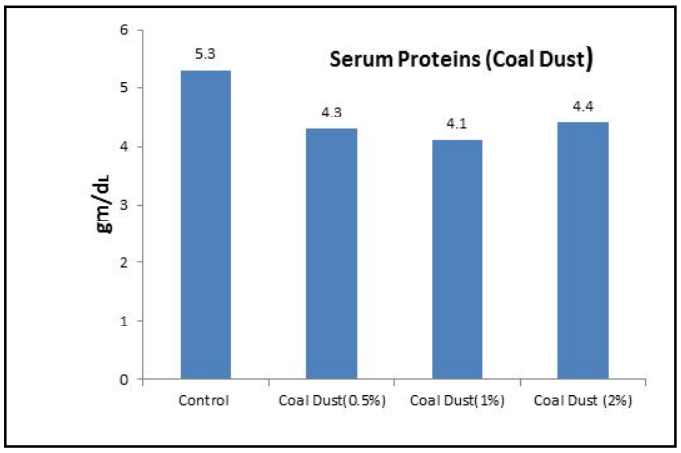

Fig-1

Serum Albumin levels of *Channa striata* Exposed to (Coal Dust)

1	Control	2.2+- 0.095
2	Coal Dust (0.5%)	1.7+- 0.047
3	Coal Dust (1%)	1.65+- 0.064
4	Coal Dust (2%)	1.75+- 0.064

Table-2

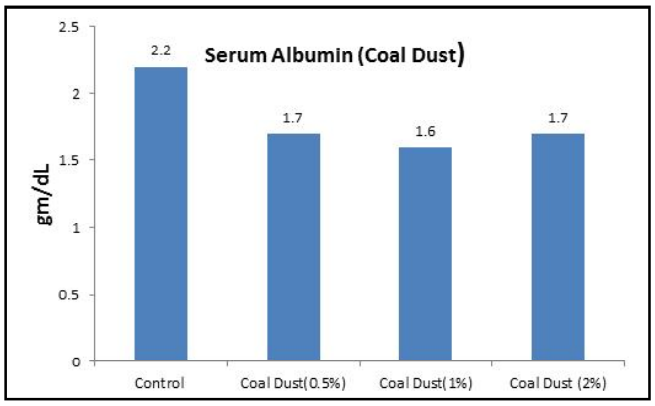

Fig-2

Serum Globulin levels of *Channa striata* Exposed to (Coal Dust)

1	Control	3.2+- 0.137
2	Coal Dust (0.5%)	2.4+-0.155
3	Coal Dust (1%)	2.2+- 0.085
4	Coal Dust (2%)	2.1+- 0.853

Table-3

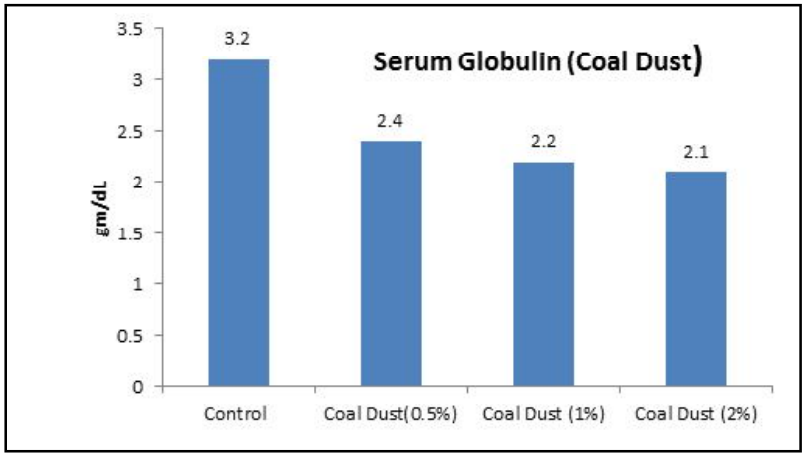

Fig- 3

Serum Protein levels of *Channa striata* exposed to (Coal ash)

1	Control	5.2+_ 0.11
2	Coal Ash(0.5%)	4.1+_ 0.131
3	Coal Ash(1%)	4.2+_ 0.095
4	Coal Ash (2%)	4.2+ _ 0.062

Table 4

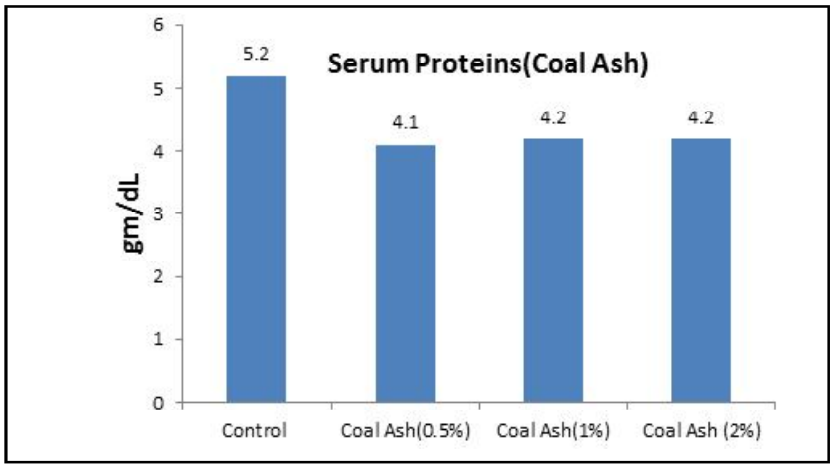

Fig-4

Serum Albumin levels of *Channa striata* Exposed to (Coal Ash)

1	Control	2.3+- 0.129
2	Coal Ash(0.5%)	1.7+-0.040
3	Coal Ash(1%)	2.1+- 0.064
4	Coal Ash (2%)	2.3+- 0.131

Table-5

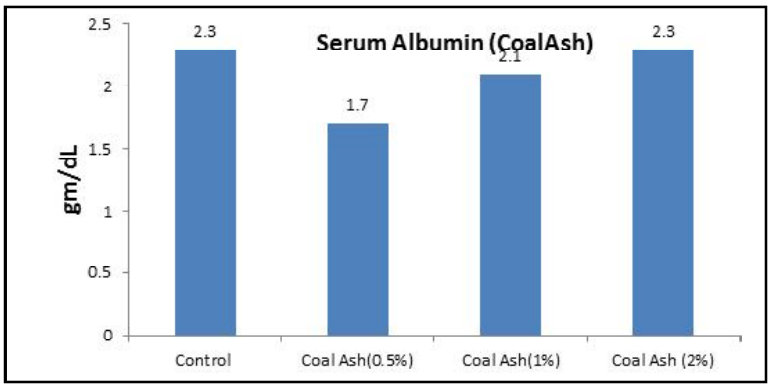

Fig-5

Serum Globulin levels of *Channa striata* Exposed to (Coal Ash)

1	Control	3.1+- 0.085
2	Coal Ash(0.5%)	2.4+- 0.155
3	Coal Ash(1%)	2.4 +- 0.085
4	Coal Ash (2%)	2.3+- 0.131

Table 6

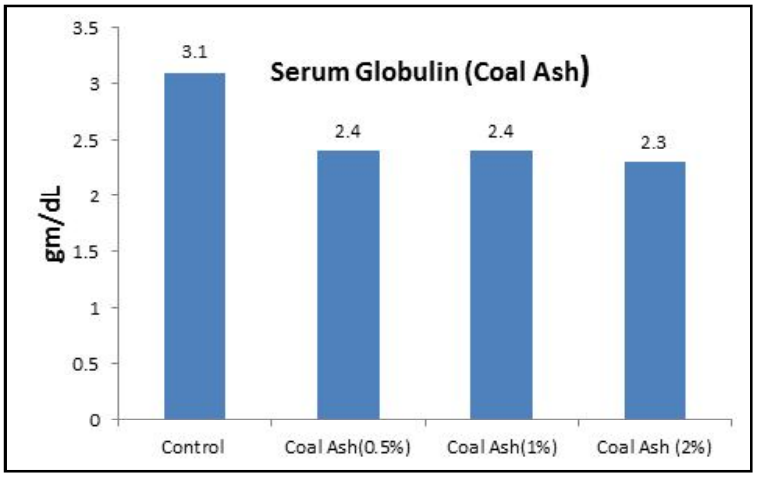

Fig-6

of Reagent and tap lightly to mix. Incubate 5 minutes at room temperature. Transfer mixtures to appropriate cuvettes and measure absorbance at 620 nm (A620).

Calculations :

Subtract the A620 of the Blank (0 g/ dL) from the A620 of each Standard and plot the A620 against standard concentrations. Use the standard curve to determine the sample albumin concentration. Conversion factors for albumin: 0.1 g/dL = 15 mM = 0.1% = 1,000 ppm.

Globulin :

Globulin levels are calculated as difference in Total serum proteins and albumin. Globulin= Total Serum Proteins- Albumin.

In the present study, Under the influence of different concentrations of coal ash and coal dust, Serum proteins, were estimated using standard biuret kit for estimation of proteins. In the present study, there was a significant decline in total serum proteins and also in serum albumin and also globulin when exposed to coal ash and coal dust under laboratory conditions. Mean and SEM was calculated. Each reading is a mean of four individual values. ANOVA was calculated using online calculator.(https://calculator-online.net/anova-calculator/) The F statistic and P value was calculated. The results were significant at ($P<0.001$). Coal pollution, either as coal dust or coal ash, has greatly impacted the serum proteins, and also albumin and globulin. (The results have been presented in tables 1-6 and illustrated as graphs (**Fig-1-6**).

In this study, apart from, total serum proteins, albumin and globulin were also estimated. *Channa striata*, was exposed to coal dust, and coal ash under laboratory conditions. Albumin is synthesized by liver hepatocytes and rapidly and is released into the bloodstream. Serum albumin, can help us to understand, the healthy functionality of the liver, as it has an ability to biosynthesize proteins and factors are vital to total body homeostasis as suggested by Chang *et. al.,*[2]; Rothschild *et.al.,*[10] and Annane *et. al.,*[1]. Albumin is the most abundant plasma protein. It accounts for ~60% of the total serum protein. Albumin plays important physiological roles, including maintenance of colloid osmotic pressure and binding of key substances such as long-chain fatty acids, bile acids, bilirubin, hematin, calcium, and magnesium. It has antioxidant and anticoagulant effects, acts as a carrier for nutritional factors and drugs, and is an effective plasma pH buffer. Serum albumin is a reliable prognostic indicator for morbidity and mortality, liver disease, nephritic syndrome, malnutrition, and protein-losing enteropathies. High levels are associated with dehydration. A general decline in serum proteins, albumin and globulin were observed in the coal treated fish compared to that of the control fish. This decline in serum proteins and lower albumin and globulin levels, clearly indicates liver damage specifically parenchymal damage as suggested by Henry[4]. In a similar study on *Channa puncatatus* by Javed and Usmani,[7], suggest that there was a necrotic tissue damage in liver when the fish were exposed to thermal plant effluent. The decrease in albumin and globulin, in *Channa,* in the present study may be attributed, to stress caused by cola pollutants, as coal ash and

coal dust, which might have caused a disturbance in the albumin / globulin levels as suggested by Javed and Usmani[7]. In conclusion it may be said, that, under the influence of coal pollutants, coal dust / ash, *Channa striata*, showed a significant decline in serum proteins, albumin and globulin, probably due to hepatocyte damage.

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