International Journal of Research and Review
www.gkpublication.in E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Tracing Out Correlation between Blood Lead and Haematological Parameters in Villagers around a Lead Mine Area

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Received: 18/09/2015 *Revised: 25/09/2015* *Accepted: 25/09/2015*

ABSTRACT

Lead concentration in different people of different age groups and sexes were analyzed by AAS around five different villages in a derelict Lead mine area in Sundargarh district of Odisha state. Different blood parameters studied included total RBC and total WBC counts, gm % of haemoglobin, haematocrit, mean. Differential counts of WBCs were also done for the respective groups. A correlation coefficient ('r') between lead content of blood and Mean values of these parameters was worked out. The r - values are found to be highly significant.

Key Words - AAS, RBC, Haematocrit, WBC, MCH, PbB.

I. INTRODUCTION

According to WHO guidelines on lead toxicity (1996), the field conditions are suitable for studying effects of endemic lead pollutants. Besides, these are several other good reasons for studying lead toxicity in field: (i) many of their disease are related to environmental quality (ii) various environmental pollutants have immunotoxic potential and (iii) many of the disease have an immune component. Moreover, there is concern about the health status which includes screening tests with conventional haematology and other functional tests.

The WHO comments on above tests and assays in field conditions indicate that- Immunological biomarkers have great potential, but have not yet been fully explored, probably owing to practical limitations of lack of specificity and predictivity. As leucocytes play a major role in specific and non-specific humoral and cellular immune responses, this parameters is used as a measure of status of the defense systems, in particular in tier-I testing Zelikoff et.al. (2002). Another possible parameter is haematocrit, however it has no known specificity for any immune function, although it may be considered as a general indicator of stress. Estimation of haemoglobin in some cases may be done. In the earlier studies it has been seen that absorption and accumulation of lead in the blood is both dose dependant as well as dependant on the period of exposure (Rout& Naik 2013). Hence blood is a good indicator of lead toxicosis and effect of lead on haematological parameters is thus evident.

II. MATERIALS AND METHODS

2.1 The Study area

The present study concentrate on blood lead level (PbB) and lead in hair (PbH) in population living around Sargipali lead mine area of Odisha which operated from 1983 to 2003 by the Hindustan Zink Limited company. Five villages around the 5km were taken as case study area for sampling as shown in the trace map. The village Bharatpur is nearby to mining site and other villages are of 1km distance from each other in between 5km radius. The village Jhimirmaul is nearby to tailing dam and river Ichha nala, which is the main drainage system of the mine.

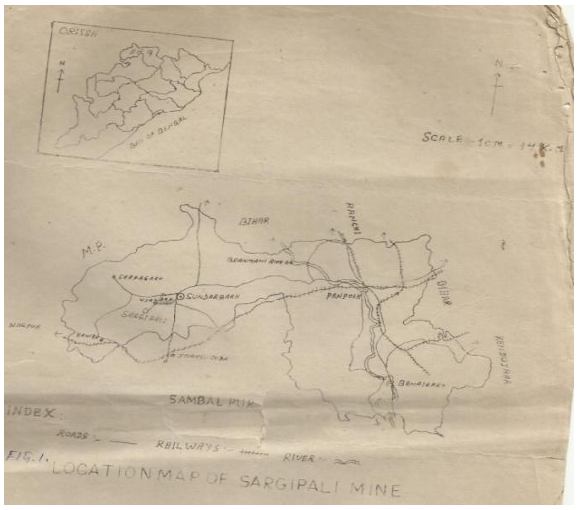


Fig.1 Trace map of the study area

2.2 Analysis of blood samples for lead:

It was followed according to Australian standard (AS2411 - 1980) for AAS. After collection the heparinized blood samples were kept at 4°C. During analysis 3ml of thoroughly mixed blood sample were immediately dispensed to the centrifuge tube. The lead in blood was made complexes with APDC by adding 0.5 ml of 2% APDC to it and extracted into 3 ml of n-butyl acetate by proper shaking. Lead was determined in the organic phase by AAS within one hour.

Calculation was done by the help of calibration curve using standard solution.

$$\text{Lead content} = \frac{\text{OD of the sample}}{\text{Standard Value}}\ \mu\text{g/gm/ml of the tissue.}$$

2.3. Haematological methods:

The methods of Sahoo (1991) for haematological studies were followed.

2.3.1. Collection of blood:

Blood from the volunteers was collected by subclavian puncture with an injection syringe (Needlle No.19). The needle and the syringe were primarily ringed by 10% sodium-heparin to prevent any chance of clotting of the blood.

2.3.2. TC of RBC & WBC:

(a) **R. B. C.:** Blood was taken out from the previously collected glass Petri dish ringed with sodium heparin sucking through micropipette up to 0.5 mark. Then Hayem's fluid was suck up to, the mark 101 and mixed thoroughly by steady rotation. One drop of blood was then passed into the cover slip of haemocytometer previously ringed and dried with spirit. After settlement it was taken for counting under microscope.

Counting was done carefully in the 1st/ 5th, 13th/ 21st and 25th smallest squares. The RBC laying on the lower and right side of the square are to be added to the total while these lying on the upper one left side were rejected.

Calculation: Total no of RBCs present in mm^3 (a) R. B. C. Blood was taken out from the previously collected glass Petri dish ringed with sodium heparin sucking through micropipette up to 0.5 mark. Then Hayem's fluid was suck up to the mark 101 and mixed thoroughly by steady rotation. One drop of blood was then passed into the cover slip of haemocytometer previously ringed and dried with spirit. After settlement it was taken for counting under microscope.

Counting was done carefully in the 1st/ 5th, 13th/ 21st and 25th smallest squares. The RBC laying on the lower and

right side of the square are to be added to the total while these lying on the upper one left side were rejected.

Calculation: Total no of RBCs present in mm^3 = No. of RBC count in 5 squares x 10000

(b) **W.B.C.:** Cleaned apparatus were used. The blood was sucked upto the 0.5 mark of the pipette/ then gentian violet solution was sucked upto the mark 11 of the pipette. The pipette was then rotated slowly so that the fluid mixed with the blood properly. Then dilution ws kept for 5 minutes/ so that whole of the WBC diluted. 1-2 drops of the fluid were removed from the pipette. The pipette was then touched just near the edge of the coverslip/ so that the counting chamber is properly flooded. The slide was kept for sometimes for settling of WBC in the fluid. Counting was then started by putting this slide under microscope. The WBCs were counted in the 4 squares of the counting chamber of the slide. The WBCs which are on the border line were not taken into consideration. WBCs were recognised by the retractile appearance and the slight coloration given to them by staining.

$$\text{Number of WBC/mm} = \frac{\text{No of cells counted x 10 x dilution}}{\text{No of squares counted (= 4)}}$$

2.3.3. D.C. of WBC: Taken a drop on the end of a clean slide, place the slide on a smooth surface holding it steady with the left hand. Held a second slide or drawing slide at 45° just in front of the drop of the blood, draw this slide in such a way that the blood spreads along its edge. Pushed the second or drawing slide forward at 45° on the first slide without exerting any pressure, a film of blood will be made on the first slide, the blood film was dried in air.

The blood film was stained with Leishman's stain or Wright's stain. After one minute distilled water added carefully to the stain. The water and stain were mixed well by sucking the mixture in and out of a pipette. After a time a greenish metallic scum forms on the surface of the mixture. Allow the diluted stain to act from 7 to 10 minutes. The stain was drained off and washed film for 10 seconds with distilled water. The film should be rose pink in colour. The slide was dried and examined it under the microscope for white blood corpuscles.

First, examined the stained film under low power of microscope to get an idea of leucocytes. Then it was examined under high power or oil immersion lens after thinly smearing the stained film with oil. In a longitudinal strip of the film count the various kinds of white blood corpuscles from top to the bottom.

Count 100 WBC noting on a paper by tally method and calculated the percentage.

2.3.4. Estimation of Hb, MCH & Haematocrit

i) ***Haemoglobin:*** Haemoglobin concentration was estimated by Sahli's haemometer. For this the graduated tube is pulled out of distilled water and then with methylated alcohol. After drying/ with the help of a dropper 2cc of N/10 HC1 is transferred into the dry graduated tube. The previously collected heparinized blood was taken into the micropipette and transferred into the graduated tube. With constant stirring distilled water was added drop wise till the colour of the experiment type matched with the side tubes. Then the blood dilution mark was noted which is the percentage of haemoglobin observed in the blood.

ii) ***Haematocrit:*** Blood collected with anticoagulant and kept in Wintrobe's tube and centrifused at 3000 rpm for 30 minutes. The volume occupied by the red cells when packed together is the PCV or haematocrit value

2.4. Statistical methods:

All the data obtained from the control and sensitive villagers were statistically analyzed as follows:

2.4.1. Pearson's Correlation Coefficient (r) : The r- values between doses of lead acetate with lead accumulation in blood as well as the lead accumulation and various parameters of the control groups & experimental were calculated with significance following Sanders (1994) and Chainy et.al. (2008).

III. RESULTS

After analysis, the blood parameters like total RBC, total WBC, and Haematocrit increase (Table 1) with increase in concentrations of blood lead, but the gm % of Haemoglobin and MCH are reduced. The total RBC content is higher in Bharatpur (BP) villagers i.e. 6.5 ±0.03, million / mm^3, Sargipalli (SP) 5.78±0.02, Jhargaon, 5.67±0.02, Jhimirmoul (JM) 6.78±0.02, Sribhubanpur 5.6±0.03. Similarly there is a dose dependant increase in total WBC content from 5.7±0.03 thousands/mm^3 to 8.5±0.02. thousands/mm^3. The gram percentage (gm%) of haemoglobin also varies from 8.5±0.05 to 11.5±0.08. The ESR/hr also varies from 10 to 30. Packed Cell Volume (PCV) is highest in Sribhubanpur (SB) i.e. 38% and lowest in Bharatpur (BP) i.e. 20%.

Table 1:Haematological parameters (Mean ± SEM) and F-values from ANOVA in blood samples of different people collected from five different villages around Sargipalli Lead mine area of Sundargarh.

Villages Studied	Blood Lead Levels (µg/ml)	Haemoglobin (Mg/ml)	TC RBC (Million)	TLC (thousands)	ESR (Mm/hr)	PCV (%)
Bharatpur (BP)	0.471±0.10 n=78	8.5 ±0.05	6.5 ±0.03	8.5 ±0.02	30.2 ±0.01	20.5 ±0.2
Sargipalli (SP)	0.323±0.08 n=94	9.5 ±0.06	5.78 ±0.02	7.8 ±0.01	24 .3 ±0.03	25.8 ±0.1
Jhargaon (Jg)	0.296±0.06 n=36	10.3 ±0.03	5.67 ±0.02	6.75 ±0.02	22.5 ±0.04	28.9 ±0.4
Jhimirimoul (JM)	0.394±0.08 n=61	9.7 ±0.02	6.78 ±0.02	6.43 ±0.01	26.4 ±0.02	24.6 ±0.2
Sribhubanpur (SB)	0.180±0.03 n=98	11.5 ±0.08	5.6 ±0.03	5.7 ±0.03	10.4 ±0.06	38.7 ±0.3

Table 2: Parson's Correlation Co efficient (r) between Blood lead (PbB) level and Haematological parameters of different people in five different villages around Sargipalli Lead mine area of Sundargarh.

Village	Hb	TRBC	TLC	ESR	PVC
BP	0.9†	0.7⊥	0.8†	0.8⊥	0.7⊥
SP	0.8⊥	0.8⊥	0.6⊥	0.7†	0.9†
JG	0.7†	0.9†	0.7⊥	0.8⊥	0.6⊥
JM	0.8⊥	0.8⊥	0.9†	0.8†	0.6⊥
SM	0.9†	0.8	0.8⊥	0.7	0.9†

Sgnificance level ⊥ 0.001, †-0.05

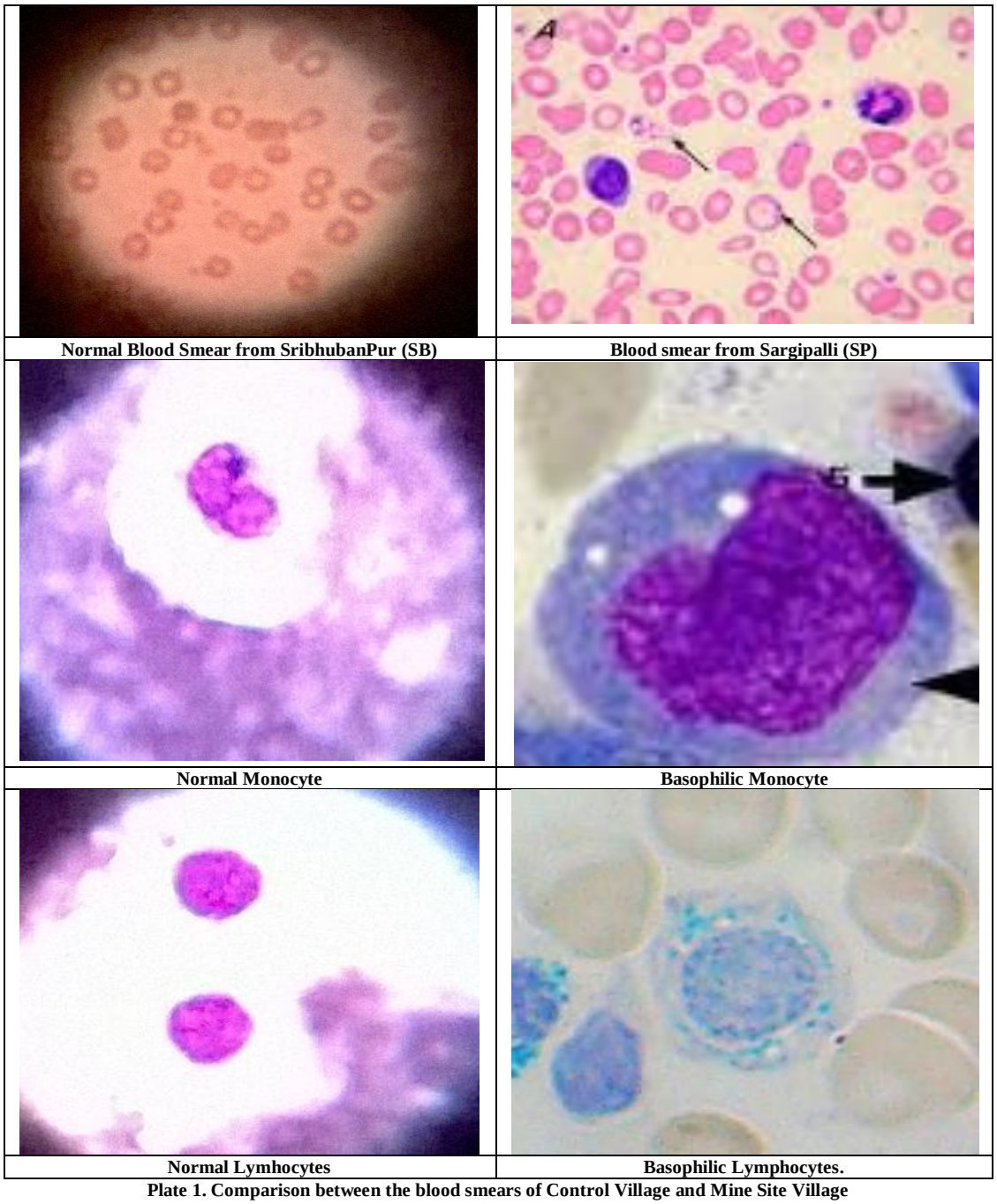
Hb- Haemoglobin, TRBC- Total RBC, TLC-Total Leucocyte, ESR- Erythrocyte Sedimentation Rate, PVC- Packed Cell Volume.

The correlation coefficient (r) between Blood lead content and different blood parameters are very highly significant (Table 2).

The differential count of WBC reveals that there is a clear case of neutropenia (Plate 1), eosinophillia basophillia, and monocytosis. The Lymphocyte percentage is almost constant. The comparison between Normal RBC of the village Sribhuban Pur (SB) and Basophilic punctuated RBC of the mine-site Village Sargipalli (SP) is shown in Plate 1. The Blood smear of the Sargipalli (SP) people show that the macrophases are granulated, the number of basophils is increased so as the immature RBCs and degraded lymphocytes.

Table 3 : D.C. of WBCs (mean % ± SEM) collected from different villages around the Lead mine area and F-values from ANOVA .

Concentration of lead acetate	D.C. of WBCs in (Mean % ± SEM)				
	N	E	B	L	M
Sribhubanpur (SB)	65 ±3.226	3 ±0.715	1 ±0.026	30 ±2.258	1 ±0.435
Jhimirmoul (JM)	55 ±2.162	12 ±1.526	5 ±0.213	20 1.216	8 ±0.126
Jhargaon (JG)	52 ±2.116	10 ±1.223	5 ±0.125	20 ±1.213	13 ±1.215
Sargipalli (SP)	50 ±5.123	15 ±1.235	6 ±1.235	15 ±2.216	14 ±2.235
Bharatpur (BP)	45 ±3.21	10 ±2.125	10 ±1.235	20 ±3.225	15 ±1.255
F-value	38.873	113.728	154.429	167.713	106.059



Normal Blood Smear from SribhubanPur (SB)	Blood smear from Sargipalli (SP)
Normal Monocyte	Basophilic Monocyte
Normal Lymhocytes	Basophilic Lymphocytes.

Plate 1. Comparison between the blood smears of Control Village and Mine Site Village

IV. DISCUSSIONS

In the previous sections it has been seen that absorption and accumulation of lead is both dose dependant as well as period of exposure. Hence blood is a good indicator of lead toxicosis and effect of lead on haematological parameters is thus evident. The principal clinical manifestations of lead toxicity on the haematopoietic system are anaemia but this occurs only with high levels of exposure (WHO 1996). The results also are very clear of this study. During subacute short-term exposure, the gm% of haemoglobin and MCH do not fall so low that the condition is said to be anaemic. Further, the increase in total count of RBC

and WBC along with cytotoxicity is a compensatory way of cell break and death during exposure to a xenobiotic. This may be a primary response in the experimental animals (Rout and Naik 2013). The increase in cell number again indicates the immaturity of the cells and their vulnerability to lytic mechanisms.

However, during chronic occupational exposures of lead exposure for prolonged period there is fall in haematocrit along with RBC and WBC number indicate a fail in respiratory mechanisms as more than 25% cell death is accounted. Haemoglobin and MCH concentration also fall in the. As per definition, a haematocrit value of less than 35% or reduction of haemoglobin to less than 50% indicates anaemic condition. Furthermore, a depressed MCH concentration show that Anaemia is microcytic (Havera et.al. 1992) in nature. This is due to an increase in plasma volume caused by disturbed water balance or inhibition "erythropoietin", the hormones for erythropoiesis or decreased erythrocyte survival. This may lead hypertension as advocated by Howard (2001). The most favourable explanation in this regard is the failure of haemoglobin synthesis as many of its steps are inhibited by lead. Lead inhibits certain enzymes like Pyrimidine -5′-nucleotidase, ACA dehydratase, ferrochelatase, C Coproporphyrin oxidase leading to basophilic stippling of RBCs and Sideroblastosis. It has also been shown that lead induces haem oxygenage activity thereby increasing the degradation of haemproteins, which may adversely affect a number of cell functions such as respiration and energy production.

The results of total and differential count of leucocytes need special attention for discussion as these cells are on the main line of the vertebrate immune system. The white blood cells count is indicative of the immune status of a person as the varieties of cells involved in the defense of the body are leucocytes. The increase in total no of WBCs in all the mine site villages may be related to the increase in functions like Phagocytosis and anaphylaxis. The results of D.C. of WBC show that in all the mine site villages, Neutrophils and Lymphocyte number decrease either with decrease in distance of exposure. In contrast Eosinophils, Basophils and Monocytes increase. This indicates the moderate to severe immune neutropenia associated with allergic status, abnormal liver disorder or chronic inflammatory processes. The reduction in neutrophil number is associated with inhibition of myeloperoxidase, Lysozymes, lactofemin and Hydrolases, which generally apply to poor defence against pyogenic bacteria. The increase in Eosinophils and Basophils increase the release of histamine perforin like Protein and C-reactive Protein, the common indices of allergy and anaphylaxis as advocated in earlier studies (Rout and Naik 1998). As the Lymphocytes are associated with the cell mediated and humoral immune responses, their decrease in number may inhibit the defense mechanism of the body. The revealed monocytes constitute of immature macrophages constituting the mononuclear phagocyte system (MPS) which serve two major functions to ingest and destroy particulate matter by opsonization when coated by complement or antibody. The other function involves the initial the recognition, Processing and presentation of antigen to the T-lymphocyte to elicit the scientific immune response. The many fold increase in number of monocytes suggest that these monocytes are either unable to mature and perform their function, or to clear the debrises due to successive cell death (as % of cytotoxicity increase) in the blood. The results are very much similar to exposure to mercury (Maheswari et.al. 2008).

REFERENCES

- Ahmad Khan, H., Malabika Sikdar-Bar, Kamlesh, B., Abdullah Wani, A., Ahmed Pervaiz, P. 2011. Lead Nitrate Induced Histopathological Changes in the Gills of the African *Clarias batrachus*. Journal of Applied Sciences Research, 7(7): 1081-1086.
- APHA. 2000. Standard methods for examination of water andwastewater. 20th Edn. American Public Health Association Washington D C. p.1976.
- Chainy, G.B.N., G. Mishra and P.K. Mohanty.2008. Biostatistics Theory and Applications. Kalyani Publishers, Ludhiana. P.353.
- Havera, S. P. Wood, S. G., and Gergi, M. M. 1992. Blood and Tissue Parameters in wild mallards redosed with lead shot. Bull. Env. Cont. and Toxicol. 49 (2) P. 238.
- Howard, Hu.2001. Poorly controlled Hypertension in a Painter with Chronic Lead Toxicity. Environmental Health Perspective, Vol.109(1) Pp.1-8.
- Khatua A.K., Rout P.C., Naik B.N. (2015). Lead contents in collected Blood samples around sargipalli Mine area Sundragarh, Odisha. Asian ResonanceVolIV (1) pp108-110
- Karnakar, N. and Sexena, R. 1990. Effect of lead acetate on erythrocyte morphology in rats. Indian J. of Exp. Biol. 28 P.1084.
- Maheswari,R. A. Devapaul, S. Muralidharan, B.Velmuruganand S.Ignaecimuthu. 2008. Haematological studies of fresh water fish *Clarias batrachus*(L) exposed to mercuric chloride. Indian Journal of Experimental Biology. Vol.2 (1)p.53.
- Mohanambal R and Purvaneswari S 2013. A study of the Acute toxicity of Lead Nitrate (Pb(NO3)2) on the fresh water fish Catlacatla (Hamilton,1862). *International Journal of Current Research. Vol. 5, Issue, 08, pp.2151-2155, August, 2013.*
- Rout PC and Naik BN 1996. Immunotoxic studies on *Clarias bachachus*$_r$ linn, during experimental Plumbism- J. Appl. Zool. Res. 7(2) pp. 185-186.
- Rout Prafulla Chandra and Naik BN 1998. Elevation of blood histataine and C-reative protien during short-term lead (Pb) exposure in *Clarias batrachus*. J.NATCON 10(2) pp.169-173.
- Rout Prafulla Chandra and Naik BN .2000. Quantitative Precipitation Tests for Anti-avidin in Lead treated Clarias batrachus Linn. during experimental Plumbism. J. Appl. Zool. Res. 11(1) pp. 48-53.
- Rout Prafulla Chandra and Naik BN 2013. Kinetics of Lead and bio-concentration factor (BCF) in different tissues of *Clarias batrachus* during experimental Plumbism. IJSRP. Vol.3 (8) pp 325-340.
- Sahoo, R.C. 1991.Studies on growth, feed efficiency and certain blood parameters in the catfish *Clarias batrachus* Linn, feed on diets varying levels of animal proteins. MFSc. Thesis. OUAT, Bhubaneswar, India.
- Sanders, D.H.1994. Staststics: A fresh Approach, Mc Graw Hill Publication, New york.
- Shamshun Nehar, Chandan Kumar, Kavita Mishra and Mamta Kumari, 2010. Lead induced alteration in blood profile of air breathing catfish, *Clarias batrachus* linn. The Bioscan:Special issue, Vol. 2.
- Talwar, G.P. (Editor) 1983: A hand book of practical Immunology. Vikas Publishing house Ltd. New Delhi.
- WHO.1996. Principles and methods of assessing direct immunotoxicity associated with exposure to chemicals. Environmental health criteria 180. Published by WHO, Geneva.
- Zelikoff, J.T., E. Karlson,Y. Li, A Raymond, J. R. Bealman and M. Anderson.2002. Immunotoxicity Biomarkers in Fish: Development, Validation and Application for Field Studies and Risk Assessment. Journal of Neurology. Vol.8 (2) pp.110-120.

How to cite this article: Khatua AK, Rout PC, Naik BN. Tracing out correlation between blood lead and haematological parameters in villagers around a lead mine area. Int J Res Rev. 2015; 2(9):555-561.

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