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Effect of Environmental Heavy Metal Pollution on Metabolic Profile of Buffaloes in Ludhiana

Pranesh V. Yeotikar, Shashi Nayyar, Chanchal Singh,
C.S. Mukhopadhyay, Rajesh Jindal, Sushma Chhabra

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ABSTRACT

Background: The industrial hub of Punjab, Ludhiana is polluted with heavy metals. The dairy animals experience stress due to pollution of heavy metals as well as the hot summer season. The stressors lead to disturbance in metabolic profile and affect milk production. The present study aimed at evaluation of detrimental effect of environmental heavy metal pollution on the metabolic profile of buffaloes during summer and winter seasons in Ludhiana district of Punjab, India. A total of 100 buffaloes were randomly selected from heavy metal exposed (n=60) and control area (n=40) during summer and winter seasons.

Methods: The location of the experimental area surveyed on the basis of levels of heavy metals. The dairy farms are located in and around Ludhiana, Punjab were identified with levels of heavy metal above the permissible limits (FSSAI, 2010) *viz*. Chromium-0.05 µg /ml; Nickel-0.02 µg /ml; Arsenic-0.05 µg /ml and Lead -0.05µg /ml. The plasma samples were analyzed for metabolic profile. The water levels of heavy metals were below the permissible limits; therefore, it was taken as uncontaminated or control area.

Result: Buffaloes of heavy metal exposed areas exhibited significantly (P<0.05) higher levels of glucose, total cholesterol, triglycerides, BUN, creatinine, AST, ALT, GGT, ALP and CK levels as compared to control area. A significant (P<0.05) decrease was observed in plasma total protein, albumin, A:G ratio, BUN: creatinine ratio in the buffaloes of exposed area as compared to the control area. However, significantly (P<0.05) higher levels of plasma glucose, BUN, creatinine, AST, ALT, GGT, ALP and CK levels were observed during summer as compared to winter season in both exposed and control areas. Significantly (P<0.05) decreased levels of plasma total cholesterol, triglycerides, total protein, albumin and BUN: Creatinine ratio were observed in the buffaloes during summer as compared to winter in both exposed and control areas. The Cr, Ni, As and Pb levels showed highly significant (P<0.01) positive correlation with plasma levels of glucose, total cholesterol, creatinine, ALT, AST, CK and GGT. Highly significant positive relationship (P<0.01) was observed among plasma levels of glucose, total cholesterol, creatinine, BUN, ALT, AST, ALP, CK and GGT whereas, plasma BUN had highly significant (P<0.01) negative correlation with plasma total protein and A:G ratio considering both the areas together. Plasma levels of total protein showed significant (P<0.01) negative correlation with all other metabolic profile constituents of both control and heavy metal exposed areas. Thus it may be concluded that the metabolic disturbance in heavy metal exposed buffaloes may have been mediated by heavy metal pollution.

Key words: Arsenic, *Bubalus bubalis,* Chromium, Enzymes, Lead, Metabolic profile, Nickel, Season.

INTRODUCTION

Industrial effluents instigating heavy metals pollution creates their potential accumulation in the biological eco system which may cost a serious environmental and health issues lasting for decades (Chopra *et al.*, 2009 and Christiana and Samuel, 2013). Metals in higher concentrations pollute surface and groundwater resources and acts as toxicants for humans and animals. Generally, water level of heavy metals are increased in summer and decreased in winter (Ozan, 2015). Toxicity of environmental contaminants like arsenic and chromium and may affect global health. Arsenic and chromium are widely distributed in nature (Vimercati *et al.,* 2017). The international Agency for Research on cancer, classified the Arsenic, Cadmium, Chromium and Nickel are toxic heavy metals are known to cause cancers. The exposure of heavy metals leads to metabolic disturbances via disruption of enzymatic activities (Banfalvi, 2011). Arsenic toxicity can disrupt hepatic function due to cross linking of enzymes (Patlolla *et al.,* 2012). Even at low levels, heavy metals can upset the normal body function, by playing as endocrine disruptors, production of reactive oxygen metabolites, hindering of essential functional groups or displacing the essential metal ions from biomolecules, leading to loss or inhibition of various enzyme activities and modification of metabolism (Lavicoli *et al.*, 2009), resulting

Department of Veterinary Physiology and Biochemistry, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

Corresponding Author: Shashi Nayyar, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India. Email: shashi.0741@ gmail.com.

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in physiological or biochemical alterations in the animal. The higher concentrations of hexavalent Cr in blood may cause blood cell damage leads to functional damage of Liver and Kidney and mainly induces apoptosis (Dartsch *et al.,* 1998). Nickel exposure through contaminated water leads to dermatitis and allergy of skin and oral epithelium damage (Jacob *et al.,* 2015). The heavy metal exposure may also induce damage to blood composition, kidneys, lungs, liver and reduction in energy levels (Hajeb *et al.,* 2014). The heavy metals affect all over the body including may leads to disruption the hematology, cardiovascular, renal and neural functions (Assi *et al.*, 2016). The metals by inhibiting or interfering with enzyme function may affect gly-colysis, krebs cycle, nucleic acid metabolism, protein metabolism as well as the pentose phosphate pathway (Strydom *et al.*, 2006). The heavy metals overload in tissues and exert their toxic effects on living organismmay also affect oxidative metabolism such as glycolysis, protein and lipid profile which later cause the oxidative stress (Javed *et al.*, 2017). Keeping this in view, the present study is therefore focusedon the effect of heavy metals on the metabolic profile of buffaloes in heavy metal exposed areas of Ludhiana district of Punjab, India during summer and winter seasons.

MATERIALS AND METHODS

Ethical approval

Experimental procedures using buffaloes in this study have been conducted after approval from the Institutional Animal Ethical Committee (IAEC) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. All the research investigations withbuffalo were carried out according to the IAEC guidelines (Proceedings of XXXVII meeting vide memo No. IAEC/2016/643-675 dated 19.10.2016, Proposal no. GADVASU/2016/IAEC/37/20).

Selection of area

The basis of selection of exposed and the control area of the present study was the levels of Cr, Ni, As and Pb in the drinking water. The area having the drinking water levels of Cr, Ni, As and Pb below the permissible limits was considered as control area whereas, the area having the drinking water levels of Cr, Ni, As and Pb above the permissible limits was considered as exposed area. The permissible limit of drinking water for Chromium-0.05 µg /ml, Nickel-0.02 µg /ml, Arsenic-0.05 µg /ml and Lead-0.05 µg/ml (FSSAI, 2010) (Yeotikar *et al.*, 2018).

Experimental animals

Group I / control area

A total of 40 Murrah lactating buffaloes selected on the basis of residing in the area where the heavy metals in the drinking water were below the permissible limits (Chromium-0.05 µg /ml, Nickel-0.02 µg /ml, Arsenic -0.05 µg /ml and Lead-0.05 µg/ml) during both summer and the winter seasons were used in the experiment.

Group II / exposed area

A total of 60 Murrah lactating buffaloes selected on the basis of residing in the area where the heavy metals in the drinking water above the permissible limits (Chromium-0.05 µg /ml, Nickel-0.02 µg /ml, Arsenic -0.05 µg /ml and Lead-0.05 µg/ml) during both summer and the winter seasons were used in the experiment.

All the experiments of the study were carried out by using lactating Murrah buffaloes (age 3-9 years). They were maintained in organized dairy farms by their owners in field conditions and provided with standard diet and ad libitum water.

Temperature humidity index

Temperature and relative humidity were recorded inside the shed with the help of thermo hygrometer. Temperature humidity index (THI) of the animal shed was calculated using the formula:

$$THI = (0.81 \times Ta) + \{(RH \div 100) \times (Ta - 14.4)\} + 46.6$$

(Where, Ta = Average ambient temperature in °C and RH = Average relative humidity) (Lakhani *et al.,* 2016).

Collection of samples

Drinking water samples

Drinking water samples were collected in duplicate in acid-washed polyethylene bottles. A total of 100 water samples were collected once in a season from tube wells of different sites of the study area of Ludhiana district. The water samples were collected in the exposed area (n=60) during summer and winter season respectively. Whereas in the control area (n=40) during summer and winter seasons respectively.

Blood samples

The blood samples (5 ml) were collected in heparinized vials by jugular venipuncture from 100 buffaloes maintained at the dairy farms located in heavy metal exposed (n=60) and control (n=40) areas of Ludhiana district Punjab during summer (June - August 2017, mean THI=82.88) and winter (November - December 2017, mean THI= 64.58) seasons.

Estimation of heavy metals in drinking water and blood samples

Determinations of heavy metals were carried out using Perkin-Elmer Optima 2100 DV model ICP-OES equipped with an AS-93 autosampler which is one of the most powerful and popular elemental analysis techniques. All samples and blank solutions are measured in duplicate. Inductively coupled plasma-optical emission spectrometry (ICP-OES) (PERKINS ELMER OPTIMA 2100 DV MODEL) instrument was used for estimating the heavy metals and trace minerals with Optima's proven 32-bit Windows® software, WinLab32™. The heavy metals (Cr, Ni, As and Pb) were analyzed in digested and extracted samples. The working standards were analyzed at the beginning and end of a run and intermittently during longer runs. According to the

absorbance, the concentration was measured directly, when the sample was well within the linear working range of the standard curve. All analyses were performed in duplicate. Analytical blanks were run along with each batch of digestion set (Dhanalakshmi and Gawdaman, 2012).

Acid digestion of water and blood samples
Digestion of water samples

Drinking water samples were digested by following the method of McGraths and Cunliffe (1995) with slight modifications. The water samples (10 ml) were added 10 ml of diacid (70 ml of Nitric acid + 30 ml of Perchloric acid) and left overnight. This mixture was digested on the hot plate at 250°C until a colorless solution 1-3 ml volume appeared. The digested samples were filtered using Whatman filter paper no. 42 and made up the volume up to 10 ml with double glass distilled water.

Acid digestion of blood samples

All glass wares were first cleaned with 10% HNO_3 solution and then further washed with the distilled water and then sterilized in the hot air oven at 160°C for 60 minutes. The whole blood samples (1 ml) were added 15 ml of a tri-acid mixture (10:4:1 HNO_3, $H2SO_4$ and $HClO_4$) and left overnight. This mixture was digested on a hot plate at 250°C until a colorless solution 1-2 ml volume appeared. The digested samples were filtered using Whatman No. 42 filter paper and the volume of filtrate was made up to 10 ml with double glass distilled water (Allen *et al.* 1986).

Estimation of metabolic parameters

Plasma levels of glucose, total cholesterol, triglycerides, total protein, albumin, blood urea nitrogen, creatinine and activities of ALT, AST, GGT, ALP and CK which were determined by Autopak kits (BPC Biopac , SRL, Rome, Italy) on the fully automatic biochemical analyzer (Global 240 BPC Biosed). All determinations were performed in duplicate. Blood urea nitrogen (BUN) concentration in plasma was calculated from plasma urea concentration after dividing by 2.14. A:G ratio, BUN: Creatinine ratio and AST: ALT ratio was also calculated.

Statistical methods

The experimental data were subjected to two way analysis of variance with interactionusing SYSTAT 13 software Inc., CA, USA, ver. 13.00.05.The differences between the mean values of the parameters belonging to the experimental groups reared during summer and winter seasons were analyzed for significant differences using Duncan's multiple range post hoc test.The normality of the data and autocorrelation between the groups were tested by Kolmogorov-Smirnov test (Lilliefors) and Durbin-Watson D-Statistic, respectively. Pearsonian correlation among the metabolic profile parameters was determined.

RESULTS AND DISCUSSION

The mean values of glucose, BUN and creatinine were significantly (P<0.05) higher in the exposed area as compared to control area during both summer and winter seasons (Table 1). The levels of total cholesterol, triglycerides, albumin and globulin were significantly (P<0.05) higher in buffaloes of the exposed area during both the seasons. However, the levels of total cholesterol, triglycerides, albumin and globulin were significantly (P<0.05) lower in buffaloes of the exposed area during the summer as compared to winter season. The levels of total protein, albumin and BUN: Creatinine ratio were significantly (P<0.05) lower in buffaloes of the exposed area as compare to control area and during the summer as compared to winter

Table 1: Metabolic profile (mean ± S.E.) of buffaloes of control and exposedareas during different seasons.

Blood biochemical constituents	Control group (n=40)		Exposed group (n=60)	
	Winter	Summer	Winter	Summer
Glucose (mg/dL)	54.24^{a}±1.46	72.64^{b}±1.95	84.93^{c}±0.86	96.41^{d}±0.69
Total cholesterol (mg/dL)	103.04^{a}±0.67	92.49^{b}±1.59	136.81^{c}±1.37	123.87^{d}±2.13
Triglycerides (mg/dL)	16.41^{a}±0.65	13.66^{b}±0.43	23.65^{a}±0.78	19.39^{c}±1.44
Total proteins (g/dL)	7.58^{a}±0.12	5.57^{b}±0.09	5.74^{b}±0.10	5.07^{c}± 0.06
Albumin (g/dL)	3.44^{a}± 0.07	2.96^{b}±0.06	1.63^{c}± 0.03	1.47^{b}±0.06
Globulin (g/dL)	3.99^{a}±0.09	2.61^{a}±0.11	4.12^{b}±0.12	3.59^{c}±0.07
A:G Ratio	0.93^{a}±0.02	1.24^{b}±0.07	0.42^{c}±0.02	0.43^{b}±0.03
BUN (mg/dL)	26.61^{a}±0.53	31.08^{b}±0.47	31.97^{c}±0.34	37.47^{d}±0.37
Creatinine (mg/dL)	1.28^{a}±0.01	1.57^{b}±0.04	2.32^{c}±0.01	2.76^{d}±0.02
BUN: creatinine ratio	20.76^{a}±0.43	20.14^{b}±0.43	14.65^{a}±0.14	13.64^{c}±0.20
AST (U/L)	91.71^{a}±1.98	108.21^{b}±2.49	129.15^{c}±2.70	137.62^{d}±3.80
ALT (U/L)	29.84^{a}±0.89	41.40^{b}±1.03	45.32^{c}±1.11	53.92^{d}±1.00
GGT (U/L)	20.72^{a}±1.80	25.35^{b}±0.97	36.98^{c}±0.63	43.07^{d}±0.66
ALP (U/L)	150.50^{a}±8.38	205.19^{b}±4.27	247.79^{c}±12.46	281.68^{d}±13.52
CK (U/L)	5.34^{a}±0.39	6.95^{b}±0.44	9.20^{c}±0.20	12.12^{d}±0.15

Superscripts a,b,c and d Means with no common superscripts are significantly different (P<0.05) within each row between different seasons vis-à-vis groups.

season in both the areas. The A: G ratio values were significantly (P<0.05) lower in exposed than control area; however, significantly (P<0.05) higher during summer than winter season in both the areas.

Pearson's correlation between heavy metals and blood biochemical constituents

The Cr, Ni, As and Pb levels showed highly significant (P<0.01) positive correlations with plasma levels of glucose, total cholesterol, creatinine and BUN whereas, they showed highly significant (P<0.01) negative correlations with total protein, albumin and A: G ratio considering both the areas together (Fig 1).

Pearson's correlation among blood biochemical constituents

Highly significant (P<0.01) positive correlations was observed among plasma levels of glucose, total cholesterol, creatinine and BUN. However, plasma levels of total protein, albumin and A:G ratio showed highly significant (P<0.01) negative correlations with plasma glucose and total cholesterol. Total protein and A: G ratio showed highly significant (P<0.01) negative correlation with creatinine and BUN considering both the areas together (Fig 2).

Pearson's correlation among plasma enzyme profile

The ALT levels showed highly significant (P<0.01) positive correlation with AST, ALP, CK and GGT. However, AST levels showed highly significant (P<0.01) possitive correlation with CK and GGT considering both the areas together (Fig 3).

Pearson's correlation between plasma enzymes profile and blood biochemical constituents

Highly significant positive correlation (P<0.01) was observed between plasma levels of ALT, AST, CK, GGT, glucose, total cholesterol, creatinine and BUN (Fig 3). However, plasma levels of total protein and albumin showed significant (P<0.01) negative correlation with all other constituents of metabolic profile studied considering both the areas together

The elevated levels of glucose in metal exposed buffaloes may be due to disturbed glucose metabolism. Arsenic and presence of heavy metals caused beta cell dysfunction may be arrested GLUT4 (Kazi *et al.*, 2009 and Chen *et al.,* 2009). The glucocorticoid system may be hampered by exposure to heavy metals and this system plays important role in carbohydrate, lipid and protein metabolism (Kaltreider *et al* 2001). The significantly (p<0.01) higher values of total cholesterol and triglycerides in heavy metal exposed buffaloes of current study have been observed by other workers Hanan, 2013 and Vaseem, 2013 may be due to disturbance of lipid metabolism and liver dysfunction. The higher values of these metabolites may be due to changes in lipid metabolism leads to impairment of these crucial pathways (Javed *et al.,* 2017).

Increased plasma glucose, total cholesterol, triglycerides with decreased plasma protein and albumin in arsenic and lead exposed cattle and mice have been recorded by El-Nekeety *et al.,* 2009, Rana *et al.,* 2010 and Mohajeri *et al.,* 2014; which is in agreement with the results of the present study. Bar-anowska Bosiacka *et al.,* 2000 reported that metals like lead affect numerous enzymes activities thus inhibiting of enzymes of glycolysis, haem and globin synthesis whichinturn influence the metabolism of erythrocytes this fact is in agreement of the present observations *viz;* alterations of glucose and total protein in heavy metal exposed buffaloes. High ambient temperature can affect the blood glucose and total cholesterol levels as physiological adaptation mechanisms in dairy animals. The reduced total cholesterol levels during the summer season in both the groups may be due to a reduction in acetate concentration which is the precursor of cholesterol synthesis (Patel *et al.,* 2016). Significantly (p<0.01) decreased total protein in heavy metals exposed buffaloes may be due to

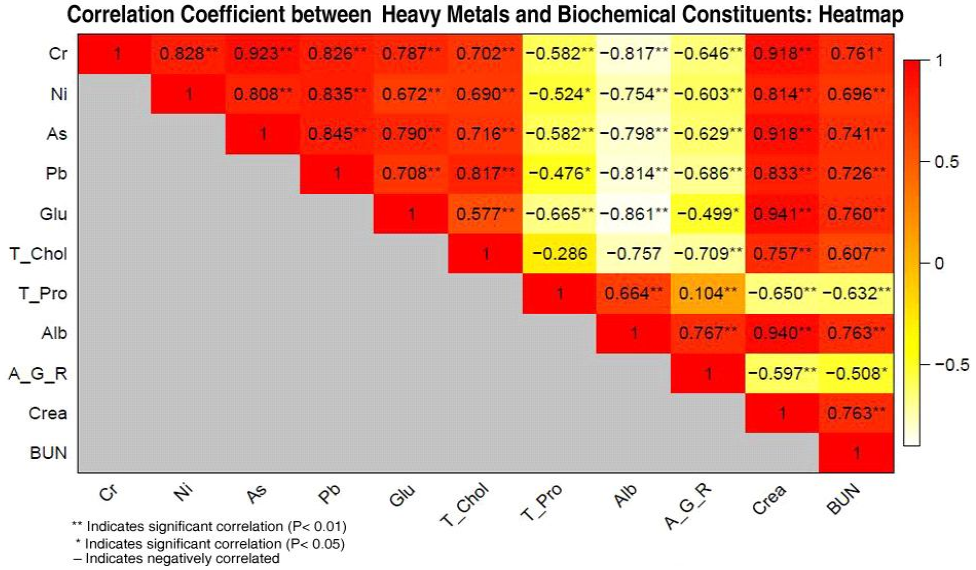

Fig 1: Correlation coefficient between heavy metals and blood biochemical constituents metabolic profile.

toxic effects of these metals on protein biosynthesis and metal salts reduce the plasma protein content is in agreement with the response observed in fish to heavy metal toxicosis by Panigrahi *et al.,* 2016. The reduced levels of total protein, albumin and globulin during the summer season in both the groups in our findings are in accordance with the findings of Dangi *et al.*, 2012 *i.e*. a significant decrease in total protein, albumin and globulin levels during heat stress in goats. These reduced levels may be due to the heat shock which increases plasma volume and decreases the protein concentrations of plasma. The reduced levels of albumin in the present experiment may be due to the liver dysfunction because of heavy metal toxicosis as albumin is produced by liver, the same observed by Javed *et al.,* 2017 in fish exposed to heavy metal loaded water.

It means there may be a source of pollution that influences the metabolic pathways extensively. Total cholesterol, total protein and albumin levels were negatively correlated ($p<0.01$) with all other metabolic profile parameters studied in heavy metal exposed areas. This may be due to the effect of heavy metals on amino acid anabolism. Heavy metal pollution may have interactive effect on gly-colysis, Krebs cycle, nucleic acid metabolism, protein metabolism, pentose phosphate pathway and Fatty acid synthesis, glycogen synthesis (Strydom *et al.,* 2006). The

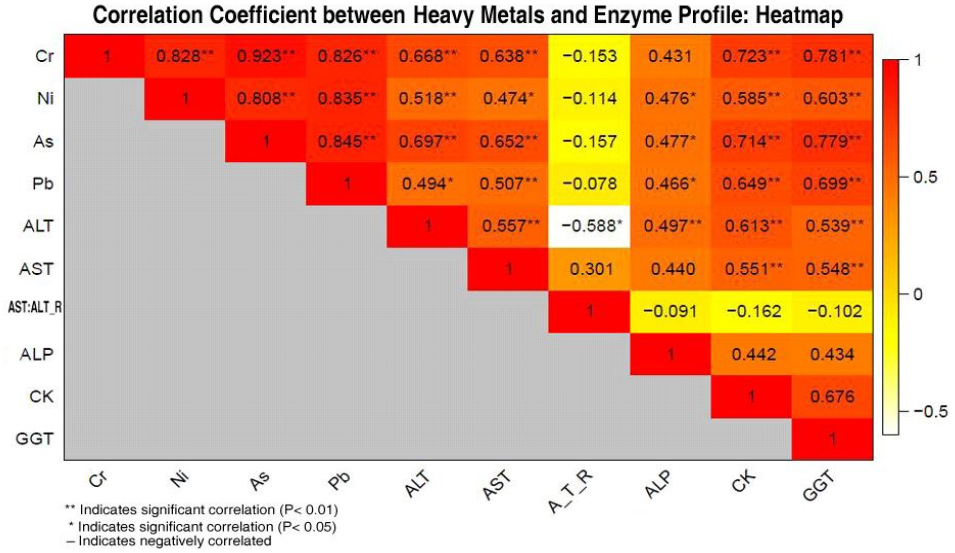

Fig 2: Relationship between heavy metals and enzyme profile in blood of buffaloes of control and exposed areas.

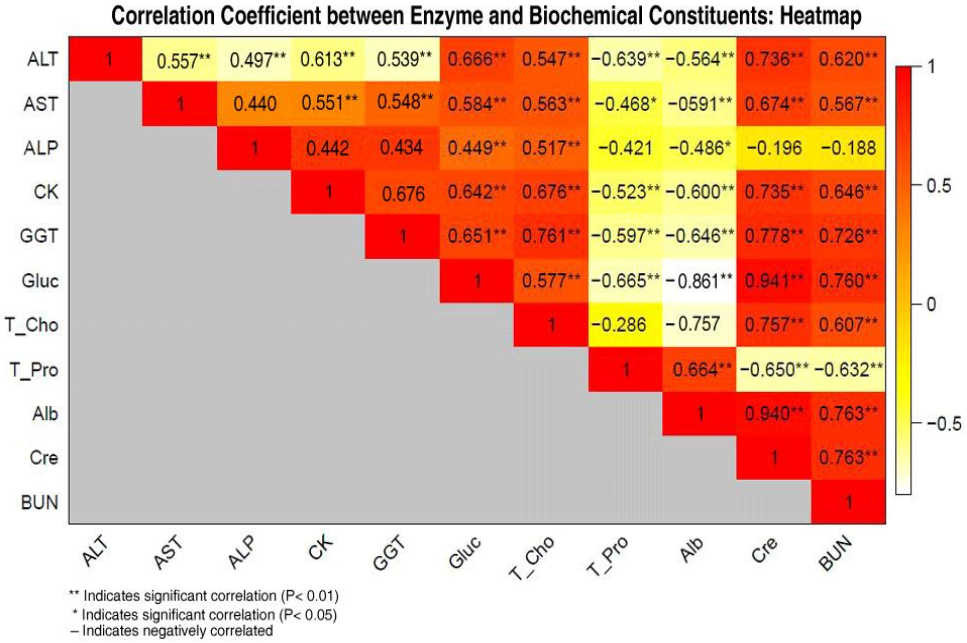

Fig 3: Relationship between enzyme profile and blood biochemical constituents of buffaloes of control and exposed areas.

elevated levels of creatinine and BUN in heavy metal exposed buffaloes may be possibly due to nephrotoxic metals like Pb and As (Maxie, 1993). The acute heat stress in young and adult Murrah buffaloes induces the changes in blood metabolites and enzymatic reactions; this may be due to adaptations of the animals to changing climate variables and environmental temperature (Haque *et al.,* 2015, Chaudhary *et al.,* 2015 and Mazuzullo *et al.,* 2014) which support the present results. The elevated levels of creatinine and BUN in heavy metal exposed buffaloes may be due to lead and arsenic which are nephrotoxic metals (Maxie, 1993).

In the present study, significantly higher levels of AST, ALP activities and reduced plasma levels of total protein and albumin clearly suggest the hepatic dysfunction in cattle with higher blood metal levels. Chand *et al.*, (2017) observed the same result in biochemical profile in cattle around industrial effluent contaminated area of kali river of Meerut city. Lead induced hepatic dysfunction in cattle was revealed by the elevated activities of AST, ALP as biomarker for liver functions and synthesis of albumin largely depends on the liver function status (Flora *et al.*, 2012). The significant increase of AST, ALT activity and decreased albumin is in agreement to those results. In hepatocellular liver diseases due to defective albumin synthesis there may be hypoalbuminemia (Chand *et al.*, 2017). Increased ALP and GGT activity observed in the buffaloes naturally exposed to arsenic contamination might be due to hepatic dysfunction (Dash *et al.,* 2016) which is in concurrence with the present study. The elevated activity of GGT is indicative of increased exposure to environmental xenobiotics (Koenig and seneff, 2015). The increased CK activity in cattle with various muscle injuries may be associated with metal toxicity (Kaneko *et al.,* 1997 and Aslani *et al. (2012)* and Dash *et al.,* 2016). Nickel and lead are cardiotoxic metals (Van Vleet and Ferrans, 1986). Highly significant positive relationship ($P<0.01$) was observed among the levels of glucose, total cholesterol, triglycerides, creatinine, AST, ALT, ALP, CK and GGT in heavy metal exposed areas. In the present study, all the enzymes analyzed showing seasonal effect during summer. The assessment of enzyme concentrations in plasma or serum interpret the physiological mechanisms as metabolic regulators during stressed conditions and their levels in serum reflects the metabolic activities (Patel *et al.,* 2016). In contrast to our findings, Helal *et al.,* 2010 observed the significantly reduced activity of ALP in heat stressed goats. Results similar to the present study were also observed in goats and found that increased levels of ALT during heat stress whereas, in contrast, no changes observed in AST concentrations in these goats (Sharma *et al.,* 2011). CK activity is higher in the plasma in response to the stress and /muscle damage (Gwaze *et al.,* 2012).

CONCLUSION

The heavy metal exposed buffaloes during summer season shows the elevated metabolic activities may be due to heavy metal pollution.

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Conflict of interest

The authors declare that they do not have any competing financial interest.

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