



Plasma heavy metals and their effect on oxidative stress parameters of buffaloes inhabiting Buddha Nallah area of Ludhiana district in Punjab

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ABSTRACT

In the present study, plasma lead, cadmium, arsenic and nickel concentrations and oxidative stress parameters in buffaloes residing near Buddha Nallah region were evaluated. Blood samples of 59 buffaloes were collected for the study and plasma was separated and analyzed for heavy metal levels and for oxidative stress parameters like reduced glutathione, malonyldialdehyde and superoxide dismutase. Significant increase in plasma heavy metal concentrations was recorded in the buffaloes of the region and significant decrease in GSH and SOD and significant increase in MDA levels were also recorded in the buffaloes of the region. Samples with higher levels of heavy metals depicted oxidative damage in the animals residing near Buddha Nallah area of the Ludhiana city.

Keywords: Buffaloes, Heavy metals, Oxidative stress, Plasma

Ludhiana is an industrial city manufacturing bicycles, sewing machines and their spare parts and dyeing units. Effluents discharged from these industries are high in lead (Pb), Cadmium (Cd), Nickel (Ni) and Arsenic (As) and are being disposed-off in Buddha Nallah, a tributary of River Sutlej passing through the city, origin of which has been sealed off and no longer contains natural water. A very high concentration of heavy metals in soil, water and plants, exceeding permissible limits (Sikka 2003; Shunthoo 2010) has been recorded. Heavy metals may get into the soil and flow into water bodies which can be taken up by plants and hence human and animal exposure of these may also be through fodder or drinking water (Engwa *et al.* 2019). The ill health effects on animals being exposed to heavy metals in the environment is of great concern. Heavy metals are known to generate free radicals which may lead to oxidative stress and cause other cellular damages (Engwa *et al.* 2019). Heavy metals induce tissue damage by increasing oxidative stress in animals being exposed. Increased lipid peroxidation and inhibition of superoxide dismutase activity indicates oxidative damage (Dhaliwal and Chhabra, 2016). Aim of the present study was to analyze heavy metal status of the buffaloes residing near Buddha Nallah area of Ludhiana district and to study effect on oxidative stress parameters of the buffaloes.

MATERIALS AND METHODS

Sample collection: The study area was divided into three zones of Buddha Nallah, i.e. Zone I (upstream), Zone II

(Ludhiana city) and Zone III (Downstream) as shown in Fig 1. A total of 59 buffaloes, aged 4–7 years were randomly selected from 10 villages of different zones of Buddha Nallah area (Fig. 1). The villages were selected so as to represent different zones within the area. Blood samples from the animals were collected in sterile heparinised test tubes containing anticoagulant. The samples were centrifuged at 3,000 rpm for 30 minutes to separate plasma. The plasma samples were stored in acid washed glass vials at –10°C until analysis. Plasma (2 ml) was digested with nitric acid and perchloric acid and after digestion the volume was made to 10 ml with distilled water for heavy metal estimation by graphite furnace using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA). Detection limits of Pb, Cd, Ni and As were 0.05, 0.002, 0.07 and 0.05 ppb, respectively.

Ten animals were kept as control which were selected from an area, 30 kilometres away from Buddha Nallah area and were not exposed to pollution of Buddha Nallah.

RBC haemolysate formation and estimation of GSH, SOD and MDA: Blood samples were centrifuged at 2,000 rpm for 10 minutes and the supernatant was discarded. The sediment cells were washed with normal saline solution. The process was repeated thrice. Washed erythrocytes were haemolysed with 9 times volume of distilled water to prepare a 10% RBC haemolysate. The haemolysate was used to analyze reduced glutathione, malonyldialdehyde and superoxide dismutase.

The levels of erythrocyte reduced glutathione were assayed as per Beutler *et al.* (1963). Product of lipid peroxidation, i.e. malonyldialdehyde in erythrocytes was assayed by the method described by Placer *et al.* (1966)

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Fig. 1. Surveillance of heavy metals in buffaloes inhabiting Buddha Nallah, Ludhiana.

and the superoxide dismutase levels in erythrocytes were measured by the method of Nishikimi *et al.* (1972).

RESULTS AND DISCUSSION

The overall mean value of plasma Pb of buffaloes inhabiting Buddha Nallah area was 0.55 ± 0.08 ppm, which was significantly ($P < 0.01$) higher than the mean value of control (Table 1). The mean plasma Pb values in different zones ranged from 0.10 ± 0.01 ppm to 1.15 ± 0.09 ppm and in zone II and III these values were significantly ($P < 0.01$) higher than the control value (Table 1). Toxic plasma lead levels (0.35–32 ppm) were noticed in 42.9% buffaloes, with highest prevalence (100%) in zone III followed by zone II (26.3%) as shown in Table 2. None of the zone I samples was in toxic range. High (0.30–0.40 ppm) range of plasma Pb levels was recorded in 6.8% animals whereas 50.8% plasma samples of buffaloes samples were in normal (0.01–0.2 ppm) range. Higher levels (0.883–1.539 ppm) of Pb were observed by Somasundaram *et al.* (2005) in jersey cows after 4 weeks of exposure to fodder irrigated with sewage water. Dwivedi *et al.* (2001) also observed high lead

levels (1.43 ± 0.07 ppm) in blood of buffaloes reared near primary lead-zinc smelter in India, without clinical signs of Pb poisoning.

The overall mean plasma Cd level of the buffaloes were significantly ($P < 0.01$) higher than the mean control value (Table 1). In zone II (0.07 ± 0.02 ppm) and zone III (0.29 ± 0.03 ppm) these values were significantly higher ($P < 0.05$ and $P < 0.01$), respectively than those of control. Overall, 61% of the buffaloes had high (> 0.04 ppm) plasma Cd levels (Table 2) with highest prevalence in zone III (95%) followed by zone II (68.4%) and zone I (20%). Dwivedi *et al.* (2001) observed high blood Cd levels in buffaloes reared near lead-zinc smelter in India (0.11 ± 0.01 ppm). These values were considerably higher than those for rural cattle in India. Gaafar (2008) observed accumulation of Cd in plasma (0.018 ppm) of buffaloes grazing on berseem or reed plants in Egypt. Tomza-Marciniak *et al.* (2011) reported that Cd concentration in dairy animals from south Turkey averaged $0.0003 \mu\text{g/ml}$, which was very low than the levels observed in the present study. According to ATSDR (1999), blood Cd levels are

Table 1. Levels of heavy metals in plasma samples of buffaloes, inhabiting Buddha Nallah area of district Ludhiana, Punjab (Mean $\pm$ SE)

Parameter	Control	Normal limits ^a	Zone I (n=20)	Zone II (n=19)	Zone III (n=20)	Overall (n=59)
Pb (ppm)	0.10 ± 0.02	0.1–0.2	0.10 ± 0.01	$0.39 \pm 0.12^{**}$	$1.15 \pm 0.09^{**}$	$0.55 \pm 0.08^{**}$
Cd (ppm)	0.02 ± 0.004	0.001–0.04	0.01 ± 0.004	$0.07 \pm 0.02^{*}$	$0.29 \pm 0.03^{**}$	$0.12 \pm 0.02^{**}$
Ni (ppm)	0.001 ± 0.000	0.001–0.006	$0.002 \pm 0.000^{**}$	$0.011 \pm 0.003^{**}$	$0.075 \pm 0.010^{**}$	$0.029 \pm 0.005^{**}$
As (ppm)	0.07 ± 0.02	0.003–0.05	$0.10 \pm 0.05^{*}$	$0.14 \pm 0.05^{*}$	$0.39 \pm 0.06^{*}$	0.21 ± 0.05

*, Significant difference ($P < 0.05$) with control group; ^a, Puls (1994); **, Significant difference ($P < 0.01$) with control group.

Table 2. Prevalence (%) of heavy metals in plasma samples of buffaloes inhabiting Buddha Nallah area of district Ludhiana, Punjab

Parameter	Status*		Zone I (N=20)	Zone II (N=19)	Zone III (N=20)	Total (N=59)
Pb	Normal	0.01–0.2	19 (95%)	11 (57.9%)	–	30 (50.8%)
	High	0.30–0.40	1(5%)	3(15.8%)	–	4 (6.8%)
	toxic	0.35–32	–	5 (26.3%)	20 (100%)	25 (42.9%)
Cd	Normal	0.001–0.04	16 (80%)	6 (31.6%)	1 (5%)	23 (39%)
	High	>0.04	4 (20%)	13 (68.4%)	19 (95%)	36 (61%)
Ni	Normal	0.001–0.006	19 (95%)	10 (52.6%)	–	29 (49.2%)
	High	>0.006	1 (5%)	9 (47.4%)	20 (100%)	30 (50.8%)
As	Normal	0.003–0.05	12 (60%)	7 (36.8%)	3 (15%)	22 (37.3%)
	High	>0.05	8 (40%)	12 (63.2%)	17 (85%)	37 (62.7%)

*Puls 1994.

indicative of recent exposure to Cd but are not especially indicative of total body burden. Studies with Cd revealed that the primary route for its toxicity is depletion of glutathione and bonding to sulphhydryl groups of proteins (Jomova and Valko, 2011).

The overall mean plasma Ni value of the buffaloes (0.029 ± 0.005 ppm) was significantly ($P < 0.01$) higher than the mean control (0.001 ± 0.000 ppm) value (Table 1). The mean plasma Ni values of buffaloes of all the three zones were significantly ($P < 0.01$) higher than the control value. Similar findings were observed by Ogabiela *et al.* (2011) in cows' blood (0.04 – 5.05 ppm of Ni), grazing around industrial estate, Kano Nigeria.

Overall, 50.8% of buffaloes had high (>0.006 ppm) Ni levels (Table 2). Highest prevalence was seen in zone III (100%). Gaafar *et al.* (2005) observed that the concentrations of Ni in plasma of Friesian calves increased with increasing their contents in ration. Yazar *et al.* (2006) reported considerably high amount of Ni (0.2505 mg/l) in blood of goats in Turkey. Gaafar (2008) observed accumulation of nickel in plasma of buffaloes (0.055 ppm) grazing berseem or reed plants in Egypt.

The overall plasma As values of the buffaloes (0.21 ± 0.05 ppm) were significantly ($P < 0.01$) higher than the control value (Table 1). In the zone I (0.10 ± 0.05 ppm), zone II

(0.14 ± 0.05 ppm) and zone III (0.39 ± 0.06 ppm) these values were significantly ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively) higher than the mean control value. Overall, 62.7% of the buffaloes had high (>0.05 ppm) plasma As levels with highest prevalence in zone III (85%) followed by zone II and I (Table 2). Singh (2012) also reported higher plasma As levels in buffaloes from Mansa district of Punjab, which ranged from non detectable to 0.287 μ g/ml. Roy *et al.* (2013) reported 2–3 ppb As content in goat tissue samples collected from different districts of neighboring state, Haryana. Gaafar (2008) observed accumulation of As in plasma (0.07 ppm) of buffaloes in Egypt.

Similar trend of increase in heavy metal concentrations was noted in water samples of Buddha Nallaharea of all the three zones. Significant ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively) increase in all the heavy metals in water samples of zone I, zone II and zone III of Buddha Nallahas compared to samples of control region was observed (Table 4).

Oxidative stress was evaluated by analysing GSH, SOD and MDA levels in RBC hemolysate. The mean value of GSH in erythrocytes of buffaloes (0.69 ± 0.09 mM) was significantly ($P < 0.01$) lower than the mean control value (Table 3). The GSH in erythrocytes of buffaloes inhabiting Buddha Nallah area ranged from 0.40 ± 0.03 mM to

Table 3. Oxidative stress parameters of buffaloes inhabiting Buddha Nallah area of District Ludhiana, Punjab (Mean $\pm$ SE)

Parameter	Control	Zone I (n=20)	Zone II (n=19)	Zone III (n=20)	Overall (n=59)
GSH (mM)	1.01 ± 0.12	$0.88 \pm 0.17^{**}$	$0.78 \pm 0.06^{**}$	$0.40 \pm 0.03^{**}$	$0.69 \pm 0.09^{**}$
MDA (nmol/g Hb)	259.6 ± 45.8	$300.5 \pm 49.9^{*}$	$309.8 \pm 60.7^{*}$	$407.07 \pm 112.4^{*}$	$339.33 \pm 74.33^{*}$
SOD (U/mg Hb)	36.03 ± 5.24	$28.2 \pm 4.00^{**}$	$22.02 \pm 2.51^{**}$	$15.42 \pm 2.10^{**}$	$21.88 \pm 2.87^{**}$

*, Significant difference ($P < 0.05$) with control group; **, Significant difference ($P < 0.01$) with control group.Table 4. Levels of heavy metals in water samples of different zones of Buddha Nallah area of district Ludhiana, Punjab (Mean $\pm$ SE)

Parameter	Control	Zone 1	Zone 2	Zone 3	Overall (n=24)
Pb (ppm)	0.07 ± 0.01	$0.109 \pm 0.019^{*}$	$0.190 \pm 0.018^{*}$	$0.309 \pm 0.034^{**}$	$0.203 \pm 0.02^{**}$
As (ppm)	0.013 ± 0.005	$0.08 \pm 0.01^{*}$	$0.133 \pm 0.039^{*}$	$0.548 \pm 0.191^{**}$	$0.25 \pm 0.08^{**}$
Ni (ppm)	0.005 ± 0.002	$0.01 \pm 0.002^{*}$	$0.033 \pm 0.015^{**}$	$0.055 \pm 0.036^{**}$	$0.030 \pm 0.17^{**}$
Cd (ppm)	0.007 ± 0.002	$0.01 \pm 0.003^{*}$	$0.054 \pm 0.016^{*}$	$0.240 \pm 0.036^{**}$	$0.098 \pm 0.017^{**}$

*, Significant difference ($P < 0.05$) with control group; **, Significant difference ($P < 0.01$) with control group.

0.88±0.17 mM. According to Patrick (2006), GSH levels decrease due to multifactorial pathogenesis. Heavy metals directly interrupt the activity of enzymes and antioxidant sulphhydryl pools are deactivated. Arsenite binds with nucleophilic sulphhydryl groups and reduces GSH content in tissues leading to oxidative threat to tissue (Rana *et al.* 2012). Sandhir and Gill (1995) observed a direct correlation between Pb concentration and lipid peroxidation. Gurer and Ercal (2000) also demonstrated decreased GSH in rats chronically exposed to Pb. Cadmium exposure also disturbs GSH and metallothionein levels and may allow free radicals to attack double bonds in lipids of membranes and leads to lipid peroxidation.

The mean value of MDA (malondialdehyde) in the buffaloes (339.33±74.33 nmol/g Hb) was significantly ($P<0.05$) higher than the mean control value (Table 3). The MDA values of buffaloes of different zones of Buddha Nallah ranged from 300.50±49.90 nmol/g Hb to 407.07±112.4 nmol/g Hb. MDA is a well known lipid peroxidation indicator after Cd exposure (Shaikh *et al.* 1999). Arslan *et al.* (2011) observed three times elevation in the average MDA level of the cattle living near roads whereas Misra *et al.* (1990) observed increase in lipid peroxidation of tissues of nickel chloride treated rats. A dose and time-dependent increase in MDA production in serum treated with As, Cd, and Hg was recorded by Aflan *et al.* (2015).

The mean value of SOD in buffaloes (21.88±2.87 U/mg of Hb) was significantly ($P<0.01$) lower than the mean control value (Table 3). The SOD values of buffaloes of different zones of Buddha Nallah ranged from 15.42±2.10 U/mg of Hb to 28.20±4.00 U/mg of Hb. Similar results of decreased SOD activity were reported by Kaminski *et al.* (2007) in chicks of polluted area. Erythrocytic SOD activities get reduced in humans also as a result of prolonged Cd exposure (Uchida *et al.* 2004). SOD requires Cu and Zn for its activity and both these metal ions get replaced by Pb and decrease the activity of SOD. Cd-induced oxidative stress results in lipid peroxidation in the liver through changing the activity of the antioxidant enzyme SOD and Cd induces enzyme misfolding which results in changes of SOD activity (Wang *et al.* 2015). In another study, acute exposure to Cd and/or Pb induced toxic effects in the blood, liver and kidneys of adult Wistar rats. Oxidative stress was a major mechanism of toxicity for both metals and observed a disturbed redox status in tissues of treated rats (Andjelkovic *et al.* 2019).

In conclusion, significant alterations in plasma heavy metal concentrations were observed in the region and the alterations in oxidative stress parameters in buffaloes with high heavy metal concentrations were indicative of oxidative damage which may further lead to health complications in the buffaloes residing near Buddha Nallah area of Ludhiana district.

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