

## Effect of sulfate pollution of pulp and paper factory on some hematological and biochemical parameters of cows.

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### Abstract

Pulp and paper industry is one of the most water and energy consuming industry in the world. Waste and wastewaters are generated during pulp and bleaching processes. It can cause public health problems. The present study was established to evaluate the effect of water waste and air emission on clinical health and some hematological and biochemical parameters of cow. It was carried out on 63 female cows of mixed breed within average age from 1 to 3 years which are kept in area 3 km before and after pulp and paper factory located in Qus city belonging to Qena Governorate. Group 1: it used as control group containing 23 animals from general slaughter house of Qena city. Group 2: animals were expected to air pollution involving 19 animals from Alhella village which located before the factory 3 km. Group 3: animals were subjected to water pollution including 21 animals pollution from Elkeratia village and Alhomor & Elgaafra Village which are located after the factory by 3 to 5 km. Water samples from three localities, from draining pipe of the factory under study and from two localities of the River Nile after the factory, were collected in clear dry separate bottles, for estimation the level of Sulfates (SO<sub>x</sub>). Heparinized blood used for CBC picture. Another blood sample were collected without anticoagulant for preparation of serum to biochemical analysis. Significant increase in sulfate in water samples was estimated. Significant decrease in Hb. conc., MCV, MCH and MCHC in groups exposed to air and water pollution. Significant increase sulfate with significant decrease of copper in groups exposed to air and water pollution. In addition, there was significant increase in urea and creatinine. It could be concluded clinically reduced performance and health of growing cows in addition cow-calf pairs had little impact on calf growth or milk production, but caused small reductions in cow BW and body condition score (BCS). Decrease cow performance, milk production, and reproduction. Also, pulp and paper effluents caused significant elevation in serum sulfate with significant decrease of copper. Thus, it induced normocytic hypochromic anemia with kidneys dysfunction.

**Keywords:** Pulp and paper industry, Pollution, Environment, Cows, Sulfate, Anemia

### Introduction

Pulp and paper industry is one of the most water and energy consuming industry in the world. Waste and wastewaters are generated during pulp and bleaching processes. Additionally, 100 million kg of toxic pollutants are released every year from this industry (Cheremisinoff & Rosenfeld, 1998).

Also, gaseous pollutants are other environmental problems generated from pulp and paper industry. The major air emissions of industry come from sulfite mills (Smook, 1992). Some mortality studies have indicated increased risk of death from

respiratory disease among pulp mill workers exposed to paper dust (Jäppinen and Tola 1990; Torén et al., 1989).

Sulfate is a substance that occurs naturally in drinking water (Spicer et al., 1974). Effects of sulfite-bisulfite ions observed at high concentrations lead to alterations of platelet function and interference in metabolic formation of red-cell 2, 3-diphosphoglyceric acid, an important intermediate in regulation of oxygen delivery to the tissues (Kikugawa and Hzuka, 1972). It resulted in some hematological deterioration due to increase in

the endothelial permeability of the blood vessels (Saada et al., 2003).

Moreover, such pronounced exposure of dairy cows to higher level of sulfur gases induced hepatorenal injuries, subsequently significantly increased ALT, AST, urea and creatinine level (Kazemi-Bonchenari et al., 2014).

## Material and Methods

1 -Animals, the present study carried out on 63 female cattle of mixed breed within average age from one to three years .

The research study was performed on 63 in the study group which are kept before and after pulp and paper factory located in Qus city belonging to Qena Governorate. All animals were fed barseem, straw and corn. The animals were divided as follow. Group 1: it used as control group containing 23 animals from general slaughter house of Qena city. Group 2: animals were subjected to air pollution involving 19 animals from Alhella village which located before the factory by 3 km. Group 3: animals were subjected to water pollution including 21 animals from Elkeratia village and Alhomor & Elgaafra Village which located after the factory by 3-5 km.

2 -Samples, heparinized blood samples (10 ml) were collected from live animals in heparinized vacotainer tubes for complete blood count (CBC). Taking of the blood samples was done by vein puncture of jugular vein after cleaning the site of puncture and using vacotainer needle according to Coles.(١٩٨٦) ،

Clear separated serum was obtained by centrifugation at 3.000 rpm for 15 minutes and resulting serum used for biochemical analysis.

### Methods

Clinical examination, all animals under investigation were examined clinically in the field and in the abattoir before slaughtering according to Radostitis et al.(٢٠٠٧) ،

Hematological analysis, complete blood picture involving red blood cells count (RBCs), white blood cells count (WBCs), hemoglobin concentration (Hb.), hematocrit (Hct.), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was made. Also, differential leucocytic count including polymorph, monocytes and lymphocytes were evaluated. These parameters were performed according to the routine hematological procedures adopted by Feldman et al., (2000) by using hematological analyzer (ERMA-Japan).

Biochemical assay, colorimetrically methods used for biochemical analysis. Serum sulfate was determined according to FAO/WHO, (1999). Serum copper was determined using Jenway colorimeter model 6051 U.K by means of a test kits supplied by Salucea Dutch after method modified by Abe et al .(١٩٨٩) ،

Serum ALT and AST enzymes were determined by using (Jenway colorimeter model 6051 U.K.) colorimetric method using Bio-diagnostic kits (Bio-diagnostic Co., Dokki, Giza, Egypt) according to Reitman and Frankel, (1957). ALP was calculated via method of Belfield and Goldberg (1971). Serum total protein and albumin evaluated according to Gornal et al., (1949) and Doumas et al., (1971), respectively. Urea and creatinine were estimated by Fawcett and Scott, (1960) and Bartles et al., (1972).

## Statistical analysis

Statistical analysis was done using SAS program. It was done to compare between control and other groups. The data were presented in form of Mean  $\pm$  Standard Deviation. The difference was considered statistically significant when  $P < (0.05)$ .

## Results

Clinical signs, the animals of the concern study were suffering from loss of body weight, poor performance, decrease of milk production and emaciation.

Estimation of sulfate in water samples, there was significant increase in the level of sulfate in water samples obtained from drainage pipe than it obtained from distance one and two kilometers after it, Table 1.

Effect of sulfate on hematological finding of cows, significant decrease in hemoglobin concentration in group 2 and group 3 exposed to air and water pollution, respectively was detected when compared with control. Moreover, blood indices involving MCV, MCH and MCHC values exhibited significant decrease in in group 2 and 3 exposed to air and water pollution, respectively when compared with control. Other hematological parameters including RBCs count, Hct. values, and platelets count recorded non-significant changes when compared with control, Table 2.

Differential leucocytic counts, it showed non-significant changes in WBCs count, lymphocytes, monocytes, and polymorph of all groups either incase of air or water pollution when compared with control group, Table 3.

**Biochemical analysis**

Serum minerals, it detected significant increase in the level of sulfate with significant decrease of copper of groups 2 & 3 exposed to air and water

pollution, respectively when compared with control, Table 4 .

Liver function tests, it displayed non-significant increase in liver function enzymes involving ALP, ALT and AST in groups 2 & 3 when compared with control group, Table 5.

Protein profile, it showed non-significant increase in protein profile involving total protein and albumin values in groups 2 & 3 when compared with control group, Table 6.

Kidney function tests, it exhibited significant increase in the kidney function enzymes involving urea and creatinine in groups 2 & 3 when compared with control group Table, 7.

**Table 1: The mean values of sulfate in water samples from drainage pipe, after one km and after two km.**

location	Sulfate (ppm)
Drainage pipe	651 ppm
After one km	250 ppm
After two km	128 ppm

**Table 2: Effect of sulfate on hematological finding in different location in cows Age 1-3 years.**

Parameters	Blood parameters						
	RBC's (x10 <sup>6</sup> /mm <sup>3</sup> )	Hb. (gm/dl)	Hct. (%)	MCV (fl)	MCH (pg)	MCHC g/l	Platelets (x10 <sup>9</sup> /mm <sup>3</sup> )
Groups							
Group 1 (control)	7.44±0.9	12.49±0.8	33.19±1.9	52.68 ±2.3	17.82 ±1.7	37.0±2.4	295.8±85.9
Group 2 (air)	7.9±0.8	9.47±1.3 *	30.67±2.5	38.7 ±3.0*	11.87 ±1.6*	31.9±0.8*	290.2±96.4
Group 3 (water)	7.8±1.0	9.46±1.3 *	30.71±2.6	38.75±3.0*	11.88 ±1.6*	31.9±0.8*	288.47±91.7

\* → significant difference when compared with group 1 (control) when P<0.05.

**Table 3: Effect of sulfate on differential leucocytic count in different location in cows Age 1-3 years.**

Parameters Groups	Differential leukocytic counts			
	WBCs ( $\times 10^3/\text{mm}^3$ )	Monocytes %	Polymorph %	Lymphocytes %
Group 1 (control)	11.69 $\pm$ 1.6	17.93 $\pm$ 5.9	27.0 $\pm$ 9.4	59.0 $\pm$ 8.8
Group 2 (air)	11.79 $\pm$ 1.7	20.6 $\pm$ 5.1	30.3 $\pm$ 9.7	55.4 $\pm$ 8.3
Group 3 (water)	11.33 $\pm$ 1.9	19.5 $\pm$ 5.9	29.1 $\pm$ 9.9	56.4 $\pm$ 8.6

**Table 4: Effect of sulfate on serum sulfate in different location in cows Age 1-3 years.**

Parameters Groups	Sulfate (mg/dl)
Group 1 (control)	0.07 $\pm$ 0.06
Group 2 (air)	2.85 $\pm$ 0.1 *
Group 3 (water)	2.84 $\pm$ 0.09 *

\*  $\rightarrow$  significant difference when compared with group 1 (control) when  $P < 0.05$  %.

**Table 5: Effect of sulfate on liver function tests in different location in cows Age 1-3 years.**

Parameters Groups	Liver function tests		
	ALP (IU/l)	AST (IU/l)	ALT (IU/l)
Group 1 (control)	393.88 $\pm$ 48.8	16.46 $\pm$ 0.7	43.6 $\pm$ 0.6
Group 2 (air)	407.57 $\pm$ 46.6	16.52 $\pm$ 0.8	43.7 $\pm$ 0.6
Group 3 (water)	406.9 $\pm$ 44.2	16.49 $\pm$ 0.8	43.65 $\pm$ 0.6

\*  $\rightarrow$  significant difference when compared with group 1 (control) when  $P < 0.05$  %.

**Table 6:**

**Effect of sulfate on serum total protein (gm/dl) and albumin (gm/dl) in different location in cows Age 1- 3 years.**

Parameters Groups	Protein parameters	
	Total protein (g/dl)	Albumin (g/dl)
Group (1)	4.27 $\pm$ 0.5	3.11 $\pm$ 0.3
Group (2)	4.47 $\pm$ 0.5	3.08 $\pm$ 0.3
Group (3)	4.41 $\pm$ 0.5	3.1 $\pm$ 0.3

**Table 7: Effect of sulfate on kidney function tests; urea (mg/dl) and creatinine (mg/dl) in different location in cows Age 1- 3 years.**

Parameters Groups	Kidney function tests	
	Urea (mg/dl)	Creatinine (mg/dl)
Group (1)	11.2±2.4	0.42±0.22
Group (2)	18.57±6.0 *	1.34±0.3 *
Group (3)	19.331±6.2 *	1.33±0.3 *

\* → significant difference when compared with group 1 (control) when  $P < 0.05$  %.

## Discussion

Pulp and paper industry is one of the most water and energy consuming industry in the world. This industry uses the fifth largest energy consumer processes; approximately 4% of total energy is used worldwide. Waste and wastewaters are generated during pulp and bleaching processes. Additionally, 100 million kg of toxic pollutants are released every year from this industry (Cheremisinoff & Rosenfeld, 1998). The major air emissions of industry come from sulfite mills (Smook, 1992). It can possess public health problems. Some mortality studies have indicated increased risk of death from respiratory disease among pulp mill workers exposed to paper dust (Jäppinen and Tola 1990; Torén et al., 1989). Also, some hematological deterioration detected due to increase in the endothelial permeability of the blood vessels (Saada et al., 2003). Moreover, such pronounced exposure of dairy cows to higher level of sulfur gases induced hepatorenal injuries, subsequently significantly increased ALT, AST, urea and creatinine level (Kazemi-Bonchenari et al., 2014). So, the present study was designed to evaluate the effect of pulp and paper effluents on clinical health and some the hematological and biochemical parameters of the exposed cow .

The result showed significant increase in the level of sulfate in water samples obtained from drainage pipe than it obtained from distance one and two kilometers far from it. Animals in this study

showed clinical manifestation as it suffering from loss of body weight, decrease of milk production, emaciation and nervous manifestation. There were negative impacts of high S diets on cattle performance and carcass characteristics (Gould, 1998). The high amounts of sulfides absorbed through the rumen wall and transported to the liver may overwhelm the capacity of the liver to detoxify sulfide. Increasing sulfur concentration resulted in linear decreases in daily gain, gain to feed ratio, final weight, hot carcass weight, and dressing percentage (Loneragan et al., 2001). Where levels of sulphate exceed 0.4 % in the diet of lambs, rumen motility and dry matter intake decrease (NRC, 1985) .

From hematological aspect, the results indicated that significant decrease in hemoglobin concentration in group 2 and group 3 exposed to air and water, respectively when compared with control. Moreover, MCV, MCH and MCHC values exhibited significant decrease in in group 2 and 3 exposed to air and water, respectively when compared with control. Other hematological parameters including RBCs count, Hct. values and platelets count recorded non-significant changes when compared with control. Non-significant changes recorded in WBCs count, lymphocytes, monocytes, and polymorph of all groups either incase air or water when compared with control group. Sulfite-bisulfite ions observed at high concentrations in vitro include alterations of platelet function and interference in the metabolic

formation of red-cell 2, 3-diphosphoglyceric acid, an important intermediate in the regulation of oxygen delivery to the tissues (Kikugawa and Hzuka, 1972). It resulted in some hematological deterioration due to increase in the endothelial permeability of the blood vessels (Saada et al., 2003). Since, an ingestion of inorganic sulfate in drinking water can induce sulfhemoglobinemia and methemoglobinemia in the blood (Digesti and Weeth, 1976; Gopalachar et al., 2005). Alam and Anjum, (2003) indicated that sulphur at higher doses in feed, had adverse effects on haemogram and leukogram values, which may have grave consequences on the health and performance .

From biochemical aspect, there was significant increase in the level of sulfate of groups 2 & 3 exposed to air and water pollution, respectively when compared with control. There were non-significant increase in liver function testes and protein profile in groups 2 & 3 when compared with control group. On the contrary, significant increase in the kidney function enzymes involving urea and creatinine detected in groups 2 & 3 when compared with control group. Pronounced exposure of dairy cows to higher level of sulfur gases induced hepatorenal injuries, subsequently significantly increased ALT, AST, urea and creatinine level (Kazemi-Bonchenari et al., 2014). It was reported that sulfur induced an elevation in the serum immunoglobulins and liver enzymes, in addition to proteinuria, and hematuria associated with kidney dysfunction (Razavi et al., 2017) .

From the previous results, it could be summarized that pulp and paper effluents caused air and water pollution. It significantly elevated level of serum sulfate with significant decrease of copper. Thus, it adversely affect on animal health performance and production in addition to the hematological indices of the exposed cow. Also, it induced kidneys dysfunction resulted in with high level of serum urea and creatinine.

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## الملخص العربي

صناعة اللب والورق هي واحدة من أكثر الصناعات التي تستهلك الماء والطاقة في العالم. ويتم توليد النفايات والمياه الملوثة أثناء عمليات اللب والتبييض والتي يمكن أن تسبب مشاكل للصحة العامة. تم إجراء هذه الدراسة لتقييم تأثير نفايات المياه وانبعاثات الهواء على الصحة السريرية وبعض المعايير الدموية والبيوكيميائية للابقار. وقد أجريت على 63 بقرة أثنى من سلالة مختلطة متوسط العمر من 1 إلى 3 سنوات والتي يتم تربيتها في المنطقة 3 كم قبل وبعد مصنع اللب والورق الموجود في مدينة قوص التابعة لمحافظة قنا. المجموعة 1: تستخدم كمجموعة ضابطة تحتوي على 23 حيواناً من مجزر قنا العمومي في مدينة قنا. المجموعة الثانية: من المتوقع تأثير التلوث عليها بعدد 19 حيواناً من قرية الحلة التي تقع أمام المصنع على بعد 3 كم. المجموعة الثالثة: من المحتمل تعرض الحيوانات لتلوث المياه بعدد 21 حيوان من قرية الكيراتبية وقرية الحمر والجعافرة والثان تقعان بعد المصنع بحوالي 3 إلى 5 كم. تم جمع عينات المياه من ثلاث مناطق، من أنبوية صرف المصنع قيد الدراسة ومن موقعين لنهر النيل بعد المصنع، في زجاجات منفصلة جافة، لتقدير مستوى الكبريتات (SOx) بالمياه. عينات الدم بمضاد للتخثر استخدم لعمل صورة دم كاملة CBC. تم جمع عينات دم أخرى بدون مضادات تخثر لتحضير المصل للتحليل البيوكيميائي. وقدرت زيادة كبيرة في الكبريتات في عينات المياه انخفاض كبير في الهيموجلوبين. و MCV و MCH و MCHC في مجموعات معرضة لتلوث الهواء والماء. زيادة كبيرة في الكبريتات مع انخفاض كبير في النحاس في المجموعات المعرضة لتلوث الهواء والماء. بالإضافة إلى ذلك، كان هناك زيادة كبيرة في اليوريا والكرياتينين. والذي يمكن أن يؤثر اكلينيكيًا ويؤدي إلى انخفاض أداء وصحة الأبقار النامية بالإضافة إلى ذلك، كان هناك تأثير قليل على معدل نمو الحيوانات وإنتاج الحليب، كما تسبب في انخفاض أوزان الأبقار والحالة الجسمانية (BCS). مع انخفاض أداء الأبقار، وإنتاج الحليب، والتكاثر. أيضاً، تسبب النفايات السائلة لمصنع لب الورق والورق في ارتفاع كبير في كبريتات المصل مع انخفاض كبير في النحاس. وهكذا، فإنه يسبب فقر الدم مع خلل في وظائف الكلى.