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Level of Trace Metals and Associated Impact on Biochemical, Hematological, and Genotoxic Effects in Occupationally Exposed Waste-Collecting Workers

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ABSTRACT

The poor disposal of solid wastes can influence the health of the occupationally exposed workers, collecting the solid wastes. The present study aimed at determining the level of the trace metals (Ni, Cr, Cd, Pb) in the blood of these occupationally exposed workers to solid wastes. Furthermore, the study explored the related genotoxic, biochemical, and hematological effects of the trace metals on solid waste collection workers. The blood samples were collected from a total of 100 solid waste collection workers (experimental group) and their BMI was recorded (20.1 ± 2 kg/m^2) whereas a total of 50 local individuals were recruited as control group and their BMI was recorded (19.40 ± 1.7 kg/m^2). The concentration of metals (Ni, Cr, Cd, Pb) was observed to be significantly higher ($P \leq 0.05$) in the blood of experimental group as compared to control. The metals accumulation in the blood was in the order of Pb>Cr>Ni>Cd. The results of the hematological assessment showed a significant reduction ($P \leq 0.05$) in Red blood cells (RBCs), Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC). The Plateletes (Pt) and Mean Plateletes Volume (MPV) were found to be significantly elevated ($P \leq 0.05$) in the exposed individuals. In workers, the liver enzymatic activities was observed to be higher ($P \leq 0.05$) in terms of AST, ALT, D.Bili, T.Bili with a significant ($P \leq 0.05$) reduction in the level of ALP. Similarlty, the level of Creatinine and Urea were significantly elevated ($P \leq 0.05$) in the experimental group. However, no significant difference was observed in the micronuclei induction (genetic damage parameters) in both the groups. The exposed individuals are at risk of occupational disease development due to lack of protective devices and poor environmental conditions.

Keywords: Heavy Metals; Solid Wastes; Occupationally Exposed Workers; Bioaccumulation; Hematology; Biochemical Profiling

Abbreviations: RBC: Red Blood Cells; HB: Hemoglobin; HCT: Hematocrit; MPV: Mean Plateletes Volume, PT: Plateletes; MN: Micronelus; MCH: Mean Corpuscular Hemoglobin; HCT: Hematocrit; WBC: White Blood Cell Count; PCV: Packed Cell Volume; MCV: Mean Corpuscular Volume; AP: Alkaline Phosphatase; ALT: Alanine Aminotransferase

Introduction

The developing countries are faced with the grave challenge of solid waste management (Cruvinel, et al. [1]). Approximately 2.01 billion tons of solid waste was generated in 2016 (Kaza, et al. [2]), which is projected to increase by 70% (3.40 billion tons) by 2025 (Laso, et al. [3]). Keeping in view the current scenario of Pakistan, a huge gap lies between solid wastes generation and their proper disposal at the dedicated dumping site. For example, a study conducted by the ministry of environment of Pakistan revealed the generation of waste at the rate of 0.391 kg/capita/day, 2.73 kg/house/day, a total of 924.3 tons of waste per day, and approximately 337, 370 tons/year in Faisalabad city (EPMC, et al. [4]). Keeping in view the instantaneous growth in the population of the city, the production of solid waste is expected to double in next few years. Due to inadequate management of solid waste, various hostile impacts on health and social life of the waste collecting workers have been noticed. The solid waste places are at risk of poor quality of life in urban areas. These workers are at risk in developing areas (de Araujo, et al. [5]). Approximately 2 million individuals operate informally as waste pickers globally. These individuals are the first to suffer as a result of insufficient and poor solid waste management (Ma, Hipel, et al. [6]). The intensity and types of risk imposed depend on their working circumstances including recycling centers, warehouses, on the roads, or in garbage dumps (Gutberlet, et al. [7]). Lack of access to personal protection equipment and hygiene practices increases the individual risk factors (Abarca-Guerrero, et al. [8]).

These risk factors are associated with extreme social vulnerability and harsh conditions, with a variety of intoxicating substances such as trace metals in over permissible limits affecting their central nervous system, for instance, which further expands their vulnerabilities health-wise (Ncube, et al. [9]). During occupational exposure, the workers are subjected to different chemicals and organic agents as well as physical elements in addition to hostile situations related to their jobs. Waste collecting workers are exposed to multiple health dangers besides protection threats (Aliyu Shehu, et al. [10,11]). A variety of waste elements are generated, disposed off, and handled by waste collection workers. These waste-collecting workers are constantly exposed to these solid waste contaminants. Long-term continuous exposure to solid waste contaminations including heavy metals may lead to occupational diseases in the exposed workers (Tiwari, et al. [12,13]. Heavy metals are defined as metals with greater or greater than 5g/cm of specific gravity. The most common metals are copper, nickel, chromium, lead, cadmium mercury, and iron. Some heavy metals like nickel and iron, if low in concentration, are crucial for survival or all types of life (Leah Johnny, et al. [14]). However, even in small quantities, heavy metals like lead, mercury, and cadmium are toxic to human beings and trigger anomalies in the organism's metabolic processes, particularly in larger amounts (Manahan, et al. [15]). Exposure to these toxic metals is prevalent in the sector, where metals are used in a broad variety of methods of production.

The existence of heavy metals such as Cd and Pb, respectively, replaces Ca and Zn with competition in biological systems with binding locations (Smith-Sivertsen, et al. [16]). In a nutshell, all heavy metals in high concentrations are toxic (Zukowska Biziuk, et al. [17,18]). Human biomonitoring for toxic metals continues to receive significant attention as efforts to avoid health risks are on a continuous increase. The main objective of biomonitoring research is to assess human exposure by comparing toxic element levels measured and compared with control group levels or background values based on literature. Due to their existence in surroundings and the resemblance of certain variables associated in biochemical pathways with biochemical interaction, lead, arsenic, cadmium, and mercury, depict the features of these metals and are of specific concern. Heavy metals induce toxicity by making complexes with cellular compounds containing sulfur, oxygen, or nitrogen (Aguilera, et al. [19-21]) upon entering into the living body via drinking water, food, and air. These complexes deactivate or regulate protein or enzyme systems that cause cellular dysfunction and ultimately necrosis (Sharma, et al. [22]). The exposure to heavy metals also lead to DNA damage, which is assessed through different assays including micronelus (MN) assay. MN assay is widely adopted to assess genotixicty induced after exposure to toxic chemicals (Morita et al. 2011) (Claxton, et al. [23,24]). MN assay is considered to be a trademark of viable genotoxicity among waste-collecting workers (Albertini et al. [25,26]). The current study was aimed to investigate the blood bioaccumulation of heavy metals (Pb, Ni, Cd, Cr) and the toxic impacts of exposure to solid waste on the collection wokers in terms of their hematology, serum biochemistry, and genotixicity in Faisalabad district (Pakistan). The resulting framework aid in understanding adverse health outcomes due to poor solid waste collection practices.

Materials and Methods

Sampling sites and criteria

Study Area and Experimental Design

A total of 100 solid waste collecting workers were randomly recruited from different places of Faisalabad district volunteerly whereas 50 individuals were sampled as control group from the same study area.

Sample Selection

Criteria for the selection of blood samples involve workers in different working places of the city. The age was between 25-60yrs. All blood samples were collected from industrial or non-industrial places followed by the aseptic condition. The amount of blood sample was 5ml from the forearm kept in the median cubital vein. Samples were divided into two parts first kept into the anti-coagulated Vacutainer and the other 3ml collected in serum Vacutainer. 100 blood samples were collected from different places of Faisalabad mostly exposed and non-exposed from heavy metals from January-May 2019. Blood samples were collected by a professional phlebotomist with a sterilized disposable syringe (SHIFA disposable syringes) (Fonti, et al. [27]).

Ethical Consideration

For conducting the study, ethical approval/consent was procured from the research ethic review committee of the Government College Women University, Faisalabad. The recruited subjects were informed regarding the nature, aim, and objectives of the study verbally. Furthermore, we had their verbal consent about/for publishing this study.

Hematological Assessment

Venous blood (5 ml) was taken from the peripheral veins of each subject's arms and drained in BD Vacutainer EDTA. The hematological parameters such as red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpus-

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cular hemoglobin (MCHC), Packed cell volume (PCV), hemoglobin concentration (Hb), hematocrit (HCT), white blood cell count (WBC) and platelets counts were determined. The hematological assessment was done with a hematological analyzer (Sysmex 17 parameters XK-21 Hematological Analyzer) by following Roohi and Riaz (2006).

Serum Biochemical Analysis of Waste Collecting Workers

Serum Isolation: The whole blood sample collected in a 1.5 mL serum collection tube making sure to avoid hemolysis. The incubated the blood sample at 37 °C for 10 minutes, the centrifugation of the sample at 10,000 rpm for 10 minutes was then made. Removed serum layer transferred for kidney function analysis to a clean tube avoiding the "buffy coat" layer. The serum samples were stored at 4 °C before further analysis.

Biochemical Analysis: The serum is used for the estimation of biochemical constituents. All the serum samples analyzed for clinical chemistry. An automated clinical chemistry analyzer was used to estimate the serum enzyme activity of ALT, AST enzymes, and also for urea and creatinine. The system was initially standardized for worker's serum. To study the effects of solid waste collecting workers, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), Alkaline phosphatase (AP), T. Bilirubin, D.Bilirubin for liver function tests, and Creatinine and Urea test for kidney function was studied by using an automated analyzer (Boehringer-Mannheim, US) with reagents from the HUMAN Kits (Human Biological Diagnostic, Germany) methodology related to (Ataro, et al.).

I. Micronucleus Assay

- To study genotoxic effects, micronucleus tests were carried out on the blood sample of waste collectors and the control fellows (Garriott, et al. [28]).
- Scoring of Peripheral blood samples (100 ml/sample) were collected in vacutainers and stored at 40 C during transportation to the laboratory (about 6 hours).
- For each individual, after smear preserve from peripheral blood lymphocyte, binucleated cells were studied. To record the number of MN their criteria proposed by IAEA and by (Fenech, et al. [29]).
- Samples were processed on the same day. The assay was carried out by the method of (Fenech, 2007).

Metals Analysis in Blood

For heavy metal analysis, by wet digestion method (2:1 HNO_3: $HClO_4$), 3 ml of blood and related concentration detected with Atomic Absorption Spectrophotometer (AA 7000 F with Autosampler and Hydride Vapor Generator, Shimadzu, Japan) were analyzed by following (Riaz, et al. [30]).

Statistical Analysis

The data were analyzed using GraphPad (Version 6). The data analyzed using ANOVA, followed by post-Dunnett's test. The significance of parameters in controls and workers and post-turkey test for comparison for heavy metals Concentration (μg/g) in blood samples was employed.

Results

Health risks associated with occupational solid waste collecting workers were investigated. Body mass of occupational solid waste collecting workers was measured to study the effects of waste material on serum biochemistry parameter, hematology (Figure 1), and genotoxic effects on workers' health assessed by micronucleus assay (Figures 1 & 2 and Tables 1-3). The concentration of estimated toxic substances (Pb, Ni, Cd, Cr) in the blood of waste collecting workers was also carried out. Concentration analysis of toxic heavy metals (Pb, Cd, Ni, Cr) in the blood serum of workers and control was measured (Data presented in Figure 2). The average Pb level measured in the blood serum of workers is (6.800±2.300) higher than the average Pb concentration of control (4.450±1.100). Similarly, the average Cd level among worker's blood was observed (2.100±0.5000) higher than the average Cd concentration of control (1.400±0.3000). The average Ni level in workers (16.00±6.300) was higher than the average Ni concentration of control (8.100±2.000) found in the blood of controls. Similarly, the average Cr level in workers (18.00±7.600) was higher than the average Cd concentration of control (7.300±2.200). Long-term exposure to solid waste may imply that the working conditions, nature of the job, and the stress of the job may impact workers' health. There were slight variations in Red Blood Cells in blood samples observed among waste collection workers compared with control. The average red blood cells measured in workers were (4.953±0.2066) which were lower than the average Red blood cells (5.383±0.2095) observed in the blood of control (Data presented in Figure 3).

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Figure 1: Site of waste collection and waste collection workers.

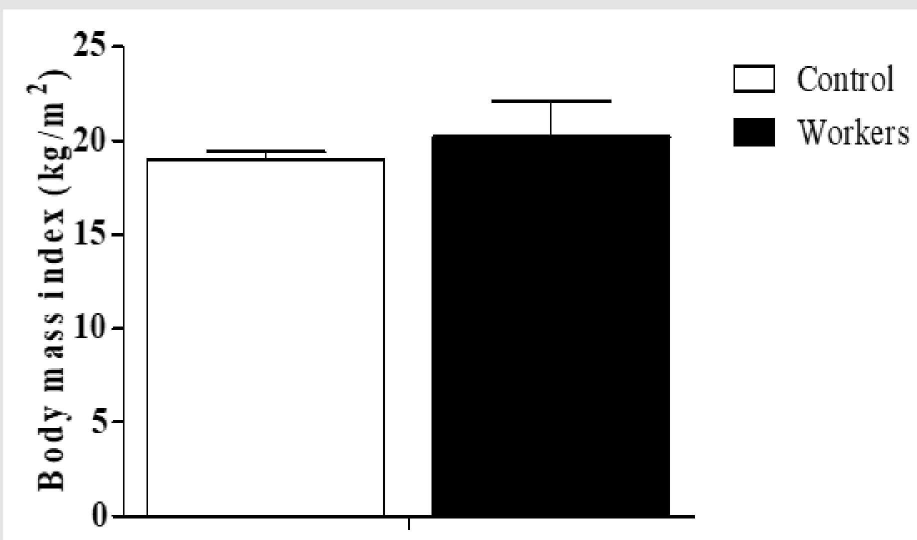

Figure 2: Body mass index (kg/m^2) of waste collecting workers and control.

Table 1: General characteristics of the subject workers.

Parameter	Subject workers	%
Age (years)		
≥20–34	66	57.9
35–44	28	24.6
45–54	20	17.5
Education level	44	38.6
Secondary school	44	38.6
Vocational school	37	32.5
Diploma degree or higher	33	28.9
Income		
<7500	76	66.7
>7000	38	33.3
Smokers		
Yes	64	56.1
No	50	43.9
Alcohol addicts		
Yes	66	57.9
No	48	42.1

Note: In workers, males were taken for study, their education level, way of living, habits, and income under consideration.

Table 2: Waste type and duration of workers are described.

Parameter	Subject workers
Leather waste-collecting workers	53
Paint workers	30
Garbage waste-collecting workers	17
Duration of work (year)	
<10	17
>10	97
Hours worked per day	
8	47
>8	68
Days worked per week	
6	83
>6	33

Table 3: Comparison of metals concentration in blood of control and waste collecting workers.

	Pb (µg/dL)		Cd (µg/L)		Ni (µg/L)		Cr (µg/L)	
	Control	Workers	Control	Workers	Control	Workers	Control	Workers
Mean	4.450	6.800	1.400	2.100	8.100	16.00	7.300	18.00
Std. Deviation	1.100	2.300	0.3000	0.5000	2.000	6.300	2.200	7.600
Lower 95% CI	4.137	6.146	0.6548	1.958	7.532	14.21	6.675	15.84
Upper 95% CI	4.763	7.454	2.145	2.242	8.668	17.79	7.925	20.16

Note: Comparison of metals concentration in blood of control and waste picker workers.

(a) Lead(Pb),

(b) Cadmium(Cd),

(c) Nickel (Ni), Chromium (Cr). Values are represented as Mean ± SD.ns $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

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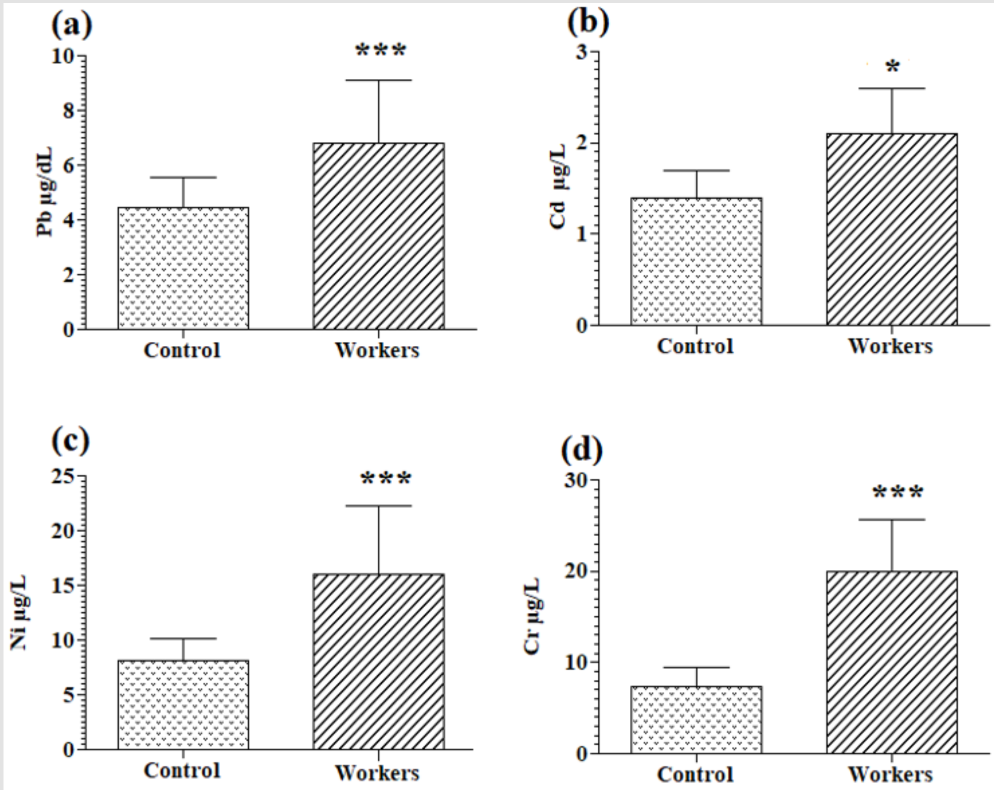

Note: Values represented as Mean ± SD. ns P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Figure 3: Comparison of Blood

(a) Lead levels (µg/dL),

(b) Cadmium (µg/L),

(c) Nickel (µg/L), and

(d) Chromium (µg/L) concentration among control group and waste collection workers.

This implies that the working conditions, nature of the job, and the stress of the job may impact the frequency of RBCs in workers. There were slight variations in Hemoglobin Concentration (g/dl) in blood samples observed in waste collecting workers compared with control. The average Hemoglobin Concentration (g/dl) measured in workers was (12.85±1.168) which was lower than the Hemoglobin Concentration (g/dl) (15.25±0.8226) observed in the blood of control (Data presented in Figure 3 and Table 3). There were slight variations in Hematocrit Concentration (g/dl) in blood samples observed in waste collecting workers compared with control. The average Hematocrit Test (HCT) level measured in the blood of workers were (41.78±2.935) which was lower than the average HCT of control (46.40±1.608) found in the blood of control (Data presented in Figure 3. Slight variations observed in MCH Concentration (g/dl) in blood samples observed in waste collecting workers compared with control. The average MCH level measured inthe blood of workers was (26.88±4.117) which is lower than the average MCH of control (33.50±2.646) found in the blood of control (Data presented in Figures 3-7. To study the genotoxic effects of heavy metals (Ni, Pb, Cr, Cd), a micronucleus assay was performed. In the present study, the micronucleus assay for white blood cells exhibited a non significant number of micronucleus cells in solid waste collecting workers compared to control. The results of the MN assay revealed no MN in peripheral blood lymphocytes of workers compared with control (Data presented in Figures 8 -10). Waste collection workers showed no genotoxic effects on workers compared with controls.

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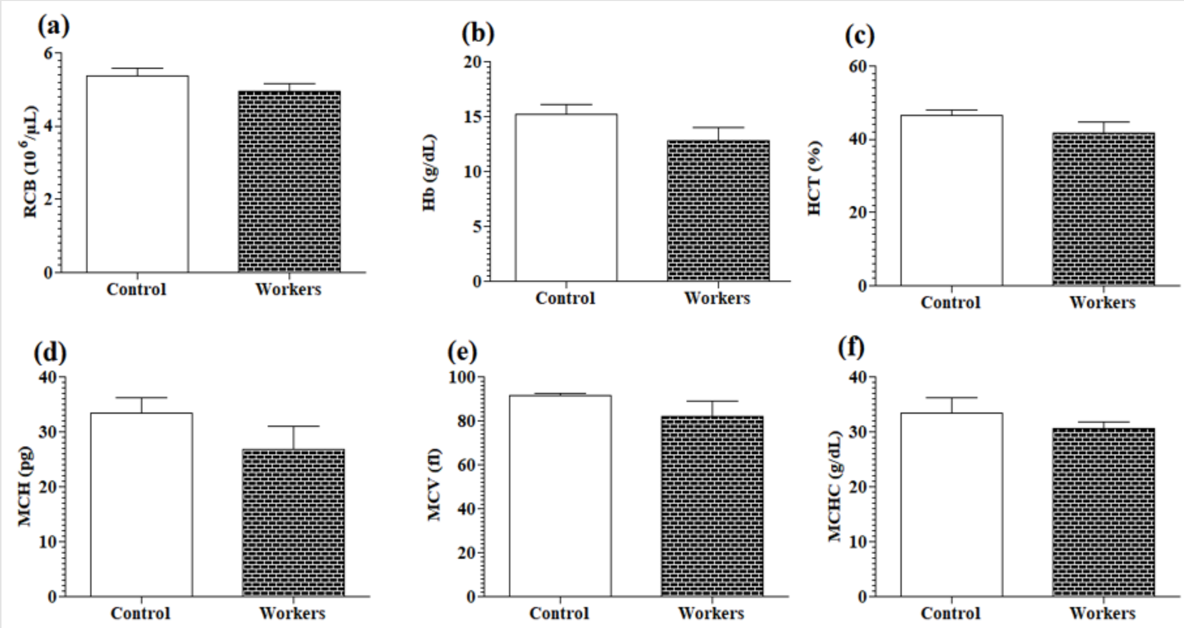
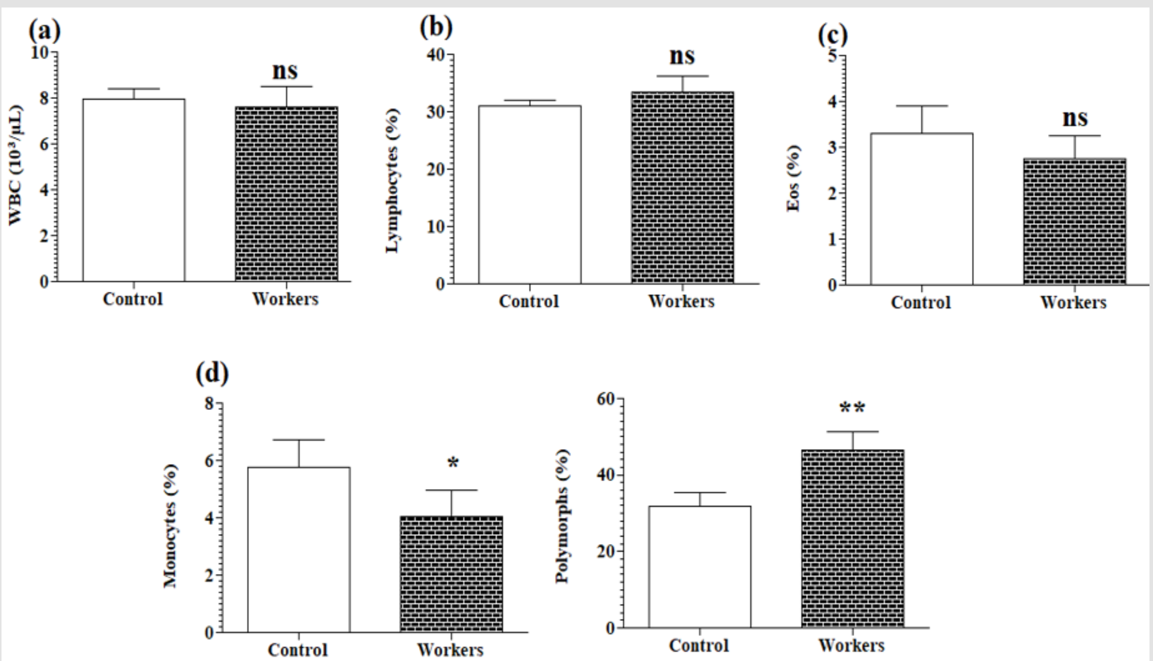


Figure 4: Comparison of Red blood cell indices of control and waste collection.



Note: Values are represented as Mean ± SD.ns P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Figure 5: Comparison of White blood cell indices of control and waste collection workers.

(a) WBC
(b) Lymphocytes
(c) Eosinophils
(d) Monocytes
(e) Polymorphs.

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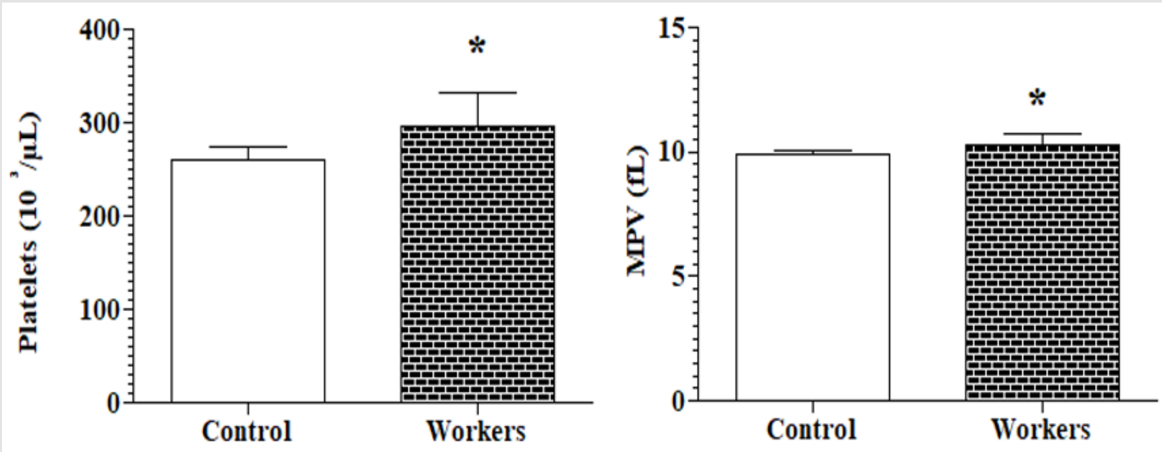
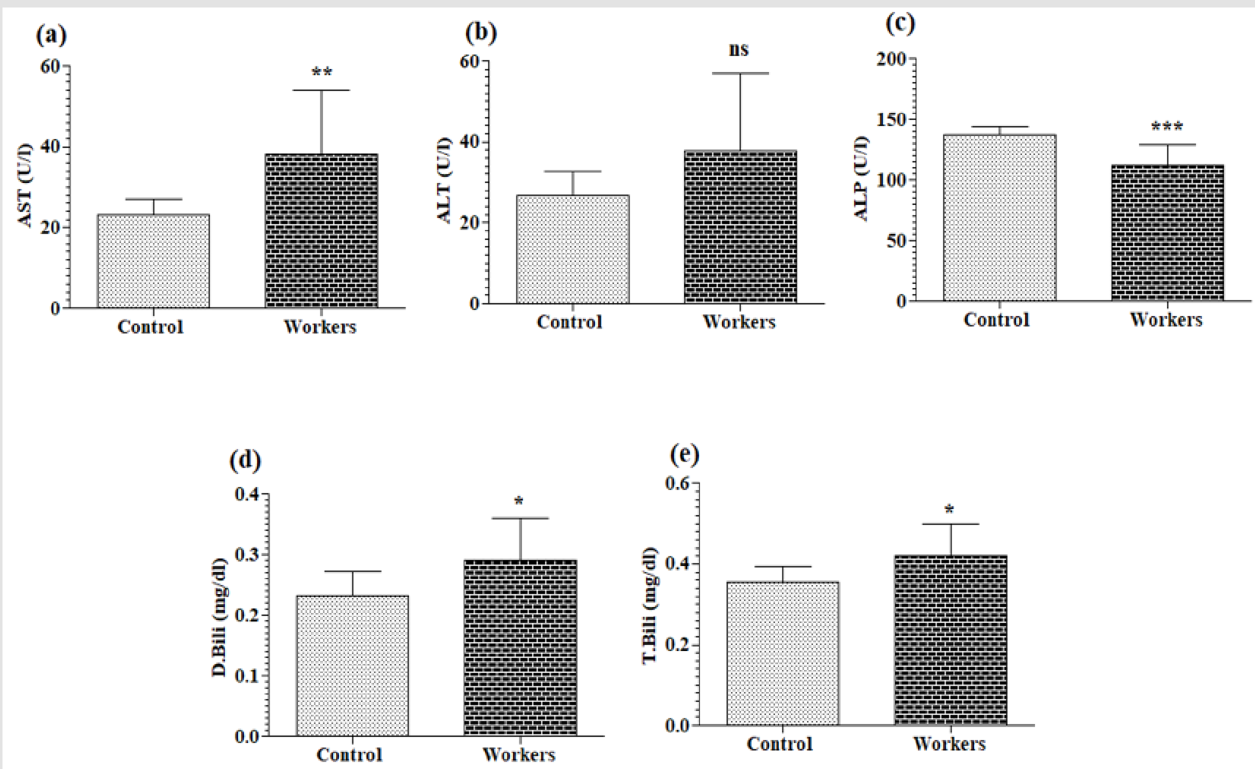


Figure 6: Comparison of Platelet indices of control and waste collection workers.



Note: Values are represented as Mean ± SD.ns P > 0.05,*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Figure 7: Comparison of liver enzymatic activities of waste collection workers and control.

(a) AST
(b) ALT
(c) ALP
(d) D.Bili
(e) T.Bili.

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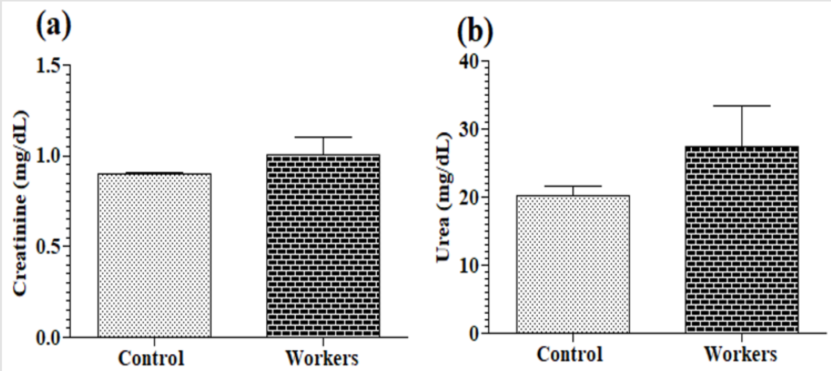

Figure 8: Comparison of kidney function of solid waste collection workers and control.

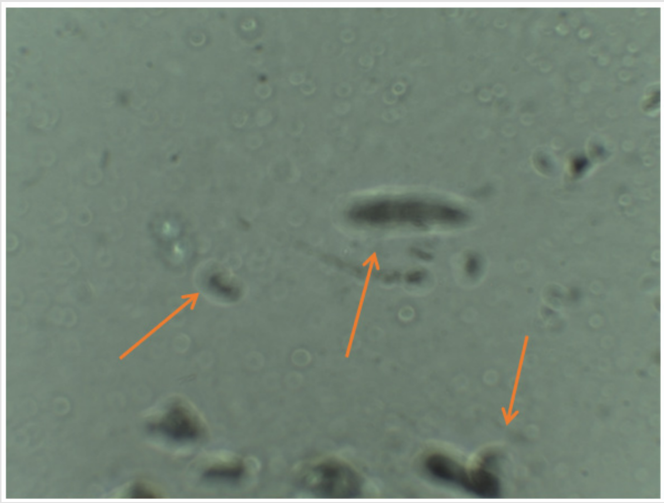

Figure 9: Micronucleus assay of solid waste collection workers.

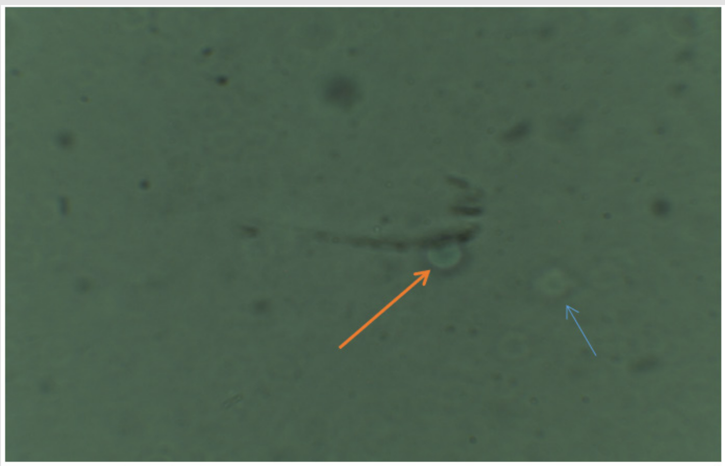

Figure 10: Micronucleus assay of Control sample.

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Discussion

(Steven Jerie, et al. [31]) identified the occupational risks associated with municipal solid waste control practices, health concerns raised about the capacity of harm from waste to the environment and the general public. The lack of health facilities among workers collecting solid waste leads to different diseases in Brazil (Silva, et al. [32]). It is also clear from the results that heavy metals also have a dramatic effect on the hematology of workers. These findings are in line with the study of those who described that alterations in blood parameters constitute one of the effects, which come up from metals exposure. In this study, RBC, hemoglobin concentration, and HCT of waste collecting workers exposed for 0.5-3years, 4-10 years, and above 10 years were significantly decreased ($p \leq 0.05$). However, the platelet count was increased compared to control (not exposed to heavy metals in wastes). (Ezeh, et al. [33]) studied hematological parameters among municipal solid waste workers compared with healthy volunteer subjects and observed that contained compounds exposed at the workplace affect the altered hematological parameters. In the present study, a statistically significant decline in the red cell count, hemoglobin, mean corpuscular hemoglobin concentration, the hematocrit value, monocyte, eosinophil, and a non-significant decrease in platelet count in solid waste collection workers observed when compared with the control group. Besides, there was a statistically significant decrease in the total white blood cell compared to the control group may be attributed to depression of the bone marrow.

There was also an increase in lymphocytes, basophil, MPV compared to controls. Our study is correlated with the study of (Wang, et al. [34]) who give a vast concept about the impact of metal toxicity on RBCs. WBCs, Platelets, MCH, MCHC, MPV, basophils, eosinophils etc. An increase in WBC observed in this present study under unhealthy situations, the level of WBC is altered; their concentration in the blood reduces especially in leukemia and acute infections (John and Hole, et al. [35]) Thus based on the present work, the increase in WBC could be from infections and inflammatory disorders since one major task of WBCs is to deliver prime resistance against possible contaminants. In the present work, a decrease in WBC eosinophils and monocytes, RBCs, in whole blood samples were found among workers occupationally exposed to heavy metals for a long period during waste collection practice. An increase in DNA damages can also be correlated with the increase in the duration of exposure to heavy metals especially among workers exposed to heavy metal hazards for more than 20 years of duration of work (Figure 1, Table 1). Metal toxicity has also an impact on the biochemistry of workers. In the present study, the effects of workplace environments focused among garbage collecting workers to estimate effects on the level of ALT, AST, ALP, AP, Creatinine, and urea. Almost all the biochemical parameters viz. alkaline phosphate, alanine aminotransferases, aspartate aminotransferases of liver were raised in the male and female workers. The total bilirubin, direct bilirubin were also higher in the workers as compared with those of non-workers.

Liver function parameters like Urea and Creatinine also increased in exposed workers than non-exposed workers of waste collecting workers. These findings are in confirmation with previous studies (Dongre, et al. [36-38]) as they also reported increase in transaminases among workers exposed to different chemicals. Effects of environmental and industrial pollution on genotoxicity have always been an important issue (Ibruljetal.,2006). (Lidiya et al. [39]) explained the Micronuclei (MN) an extra-nuclear body containing damaged and/or whole chromosome fragments that were not incorporated into the nucleus after cell division. Defects in the cell repair machinery can induce MN and accumulation of DNA damages. A variety of genotoxins reported may induce MN formation leading to cancer development, cell death, or genomic instability. The knowledge accumulated in literature on genotoxins effects precisely reflected and individual sensitivity to MN formation is discussed (Luzhna, et al. [40]). The importance of rapid MN scoring concerning genotoxic effects was also evaluated. It is also clear from the results that, heavy metals also have a not dramatic effect on micronucleus in white blood cells that cause different diseases in waste collecting workers. Over the past century, different studies by cytogeneticists have been describing the genotoxic effect of multiple exposures to heavy metals on cells and organisms (Kirsch, et al. [41]). The potential of ionizing radiation in causing damage to DNA is an example of such genotoxic influence. Different genotoxic agents use a variety of different mechanisms to alter DNA structure and affecting nuclear integrity.

For instance, mitotic spindle disrupting agents cause chromosome missegregation also reported (Elhajouji, et al. [42]), while metals have a variety of cellular targets. Therefore, genotoxic effects have been reported in many heavy metals toxicity studies (Terradas et al. [43]). Personal hygiene and behavioral risk factors were also found associated with high blood metal levels. Personal hygiene is also highly recommended for subject workers [44-126].

Conclusion and Recommendations

Heavy metal concentration analysis in waste collecting workers has been widely discussed. This study showed that the levels of chromium and lead were high in the blood of workers as compared to nickel and chromium which were moderate in their blood. Due to the high concentration of metals in blood, the effect on hematological parameters was also evident. A significant change in the White blood cells, Red blood cells, MCV, PCV, and platelet concentration were observed in the exposed individuals as compared to the control group. Similarly a significant impact of metals on liver or kidney functions of waste collecting workers was observed.

Micronucleus assay revealed minor nucleus changes. The current research demonstrated that solid waste workers are at risk of diverse health hazards ranging from infections, inflammatory diseases, leukemia, and allergic disorders. Therefore, adequate protective measures should be adopted, adequate training should be given to these workers, and they should be aware of adhering to the use of protective devices and adequate personal hygiene.

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Conflict of Interest

The authors have no conflict of interest to declare.

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