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Chemical Analysis of Heavy Metals Leaching from Old Utensils: Assessment of Cytotoxicity, Genotoxicity and Socio_Economic Awareness

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Abstract
Assessing the possible health risks caused by heavy metals leached from old cookware is crucial and requires a clear understanding of the type and amount leached . In this research quantification of selected heavy metals (Ni, Cr , Pb and Cd) , was conducted using atomic absorption spectrophotometer, leached from various types of old cookware(ceramic, aluminium, non-stick , stainless steel, granite and Pyrex ,from each type we used a single representative sample) in distilled water under different pH condition. Obtained values were compared with WHO and the Egyptian standard (2360/2007 P1 and P2) guidelines for drinking water. The genotoxicity and cytotoxicity effects were also determined . In addition, public awareness and practices regarding utensil maintenance and replacement was conducted in three distinct socioeconomic groups through a survey.
Our results revealed that highest amount of Cr leached was noted in non-stick cookware either in acid or base condition (0.186 and 0.137 mg/L respectively) followed by ceramic cookware under the same pH condition (0.1245 and 0.1142 mg/L) , these values exceeded safe WHO limits guidelines as well as Egyptian standard which represent 228% and 372 % of such
limits. Ni was higher in Non-stick and ceramic under acid condition(0.0142 and 0.0118 mg/L) and Non-stick had the highest value in alkaline pH. The amount of lead and cadmium released from cookware were minimal and often undetectable across different pH. However non-stick and ceramic cookware show low concentration of these metals under basic condition. Pyrex had almost equal amount of detected metals that found in control under all pH condition. Stainless steel cookware showed little amount of leachate under different pH . Comparison with WHO and the Egyptian standard (2360/2007 P1 and P2)
Revealed that no sample exceeded limit concerning nickel, while amount of chromium in both non stick and ceramic cookware represented about 372 & 274 % (non stick) and 249 & 228%(ceramic)that of permissible limits under acid and basic condition -in respective order. Pyrex cookware showed least amount leached which are considered closed to control and to the limits mentioned by WHO and Egyptian guideline. Low amount of lead were noted in non stick (maximum permissible limit)and ceramic cookware under basic condition. Results illustrates that old non-stick and old ceramic cookware possess various degrees of genotoxicity under acidic and basic pH compared with control. Highest percentage of viability was showed in cells exposed to sample from Pyrex cookware under acid condition, Moreover under basic pH, viability was 100% with no sign of damage in any cell. This study brought to light that Pyrex even old one is considered safe cookware. These findings highlight the serious genotoxic risk associated with old scratch Non-stick and ceramic cookware.

Keywords: Nickel, Chromuim, Lead, Cadmium Leaching solution, pH condition, Old Cookware, Comet assay.

1. Introduction
Heavy metal toxicity in food is one of alarming global universal danger . The human metabolic functions are disrupted by heavy metals by two ways: Impaired the functions of some organs like brain , kidney , liver as well as bones and they depleted essential minerals needed for biological functions , hindering their proper roles in the body [1,2] .
Nowday, society pays a lot of attention to food selection. However, unfortunately, the choice of cookware and its impact on health has not received the same level of attention [3] .
Many factors help accelerate leaching of metals from utensils like acidity of food, time of cooking, temperature, repeatability of use, and damage occurred in cookware, moreover, quality of cookware also affect leaching of heavy metals specially that made from scrap metals [4,5].
The estimated heavy metals released in food from cooking utensils sometimes exceed daily safe limits intake. Metals and alloys are widely used for the fabrication of food utensils materials. The most frequency metals used include Al, Sn, Fe, Ni ,Cr and Cu, while other toxic heavy metals can be included as impurities during manufacturing process such as As, Cd and Pb. Studies showed that migration of trace elements into food occurs by contact with metallic utensils which cause a great harmful effect on health , the most serious risk is their carcinogenic effect as some heavy metals like Cr , As , Cd, Pb, Fe , released in food from metal utensils specially scrap ones , lead to cell toxicity furthermore cancer.[6,2].

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Heavy metals leached depend on material of cookware as Ni and Li were detected in ceramics while Cr in stainless steel containers [7. kinds of cookware differ according to their composition and the materials used in their manufacture (aluminum, stainless steel, pyrex,…). The most abundant type used are that made from aluminum due to their lower cost and their effectiveness in conducting heat. Despite these advantage , it has a significant draw back because it react with food specially acid ones causing leaching heavy metals
[8 ,4] . Stainless steel utensils are known to their durability, resistance to scratches, and long life span making them a safe choice for health . They are made from nickel and chromium. If the cookware is in a good condition, the amount leached into food are minimal and poses no health risk [3] . Non-stick cookware are coated with Polytetrafluoroethylene (PTFE) which has many hazard impact to health and also stay long period in the body, it also release harmful gases when heating beside leaching in food and causing toxicity and cancer [9]. Pyrex cookware, made from pure sand (silicon dioxide), has no health hazard and are easy to clean, but have as drawback their fragility and also they are non-stick [3] .
Pots are considered durable goods due to their high price, which make them difficult for many socioeconomic levels to change periodically, and also affect cooking pots choice. Few information are available concerning cooking pots utilization, replacement as well as knowledge on their impact on health. Awareness, income and education are factors affecting cookware choices , bad choice increase risk of heavy metals leaching and affect health. Special attention are needed related to programs that help raising awareness specially in some low socioeconomic levels areas [10].
Studies about metal leaching from different cookware materials under different pH conditions has rarely been done , thus comes urgent need to study the possibility of metal leaching from different old and scrap cookware. Moreover there is a limited data about the relation between those heavy metals leached and cyto and geno toxicity.
The overall aim of this study is to establish a national baseline data about leaching of heavy metals from various kinds of old scratch cookware under different pH condition and their effect on cyto and geno toxicity as well as detect relation between women awareness concerning heavy metal leaching from cookware, education and income and their impact on health in three different socioeconomic levels.

2. Materials and Methods:

This study employed a cross-sectional survey design to explore differences across three predefined socioeconomic levels (low, middle, and high). The survey was conducted over a period of one month in Egypt (Cairo) in three different locations.

Sampling Method and Sample Size

A total sample size of 45 participants was randomly selected, 15 individuals from each of the three socioeconomic levels chosen randomly. A total of 60 individuals were approached, of whom 45 completed the survey, yielding a response rate of 75%. Non-responders were more common in the low SES group.

Socioeconomic Classification

Socioeconomic levels were classified into three distinct groups - **Low**, **Middle**, and **High SES** — based on a combination of **monthly household income**, **educational attainment**, and **occupational category.** To be classified into a socioeconomic level, participants had to meet at least **two out of the three criteria** (income, education, occupation) for that level.

Questionnaire design:

Survey schedule consist on:

*basic information about age, educational level, work, income and socioeconomic status.
*Specific questions to measure Knowledge about cooking pots choices and their impact on health and awareness regarding the use of old and scratch pots that include 10 questions total score 10 (1 mark each question) than classified to high, middle and low awareness .
*Questions about kinds of cooking pots and their advantage or disadvantage as well as cooking habits. Data were then unloaded and statically analysis. Correlation was conducted the statistical power for Pearson correlation analyses were calculated based on our total sample size (n = 45), using a two-tailed alpha of 0.05. The power to detect a medium effect size (r = 0.30) was found to be approximately 0.90, as computed using G*Power 3.1. This suggests that the study is sufficiently powered to detect correlations of moderate magnitude.

Experiments to assess heavy metals leached:

Collecting of cookware:

Various types of old scratch cook wares (Old cookware" refers to cooking vessels that have been used regularly in household settings for over three years and/or show clear signs of surface wear, such as scratches, coating degradation, or corrosion-factors that can compromise their chemical stability and increase the risk of metal leaching during food preparation).[8]
were collected from different household based on survey's information on most popular pots used. This include ceramic, aluminum, non-stick , stainless steel, granite and Pyrex (from each type we used a single representative sample one from each kind , and all of them had same diameter meaning they were uniform in size 16 cm diameter and 0.08 m_2 as surface area). Pots were well washed with liquid detergent and then rinsed with distilled water, then dried.

Sample preparation:

Distilled water adjusted on different pH level :4 as acid (same as pH of tomatoes which is about 4) , 7 as neutral and 9 as alkaline. Acid solution was prepared using HCl (acid stable under cooking temperature) while alkaline solution was prepared using sodium carbonate. For each kind of pots (ceramic, aluminum, teafal , stainless steel, granite and Pyrex size 16 cm diameter for all cookware used)750 ml of acid, neutral and alkaline distilled water choosen to prevent any sources of metal contamination to ensure that the only source of leached metals is utensils (The liquid volume ratio used is approximately 0.375 or 37.5% of the pot's estimated total volume, calculated by dividing liquid volume by pots volume)was covered then boiled for 30 min (average time for cooking rice and vegetables), cooled . Experiment was duplicated and obtained leaching solutions for each cookware were mixed together , and representative sample from each were kept in refrigerator until analyzed for chemical determination of heavy metals (leached from pots) as well as cytotoxicity and genotoxicity assessment.

Determination of heavy metals in samples:
Collected samples were filtered and then analyzed using atomic absorption spectrophotometer (PerkinElmer-AAnalyst 400) for Ni, Cd, Cr and Pb metals using specific hollow-cathode lamps for each element. The wavelengths for lead was 283.3 nm, nikel was 232 nm, cadmium was 228.8 nm and chromium was 357.9 nm. Three solutions at tested pH (4,7 and 9) served as control.

Assessment of cytotoxicity and genotoxicity:
Viability evaluation (Trypan blue exclusion assay)
After the treatment of spleenocytes by tested solution (leaching solutions from different utensil types) for 2 hrs, the cell viability was estimated using the Trypan blue exclusion method according to [11,12,13] . The cell viability was calculated by the following equation:
Cell viability %= (number of viable cells/ number of total cells) ×100

***In vitro* alkaline comet assay**
The experiment relies on the mobility of DNA or DNA fragments damaged by genotoxic agents in an agarose gel under an electric field. The DNA damage was displayed as tail like halo from circled head that represents the nucleus ; contains remain intact DNA. The treated cells (normal mouse spleenocytes) for 2 hrs suspended in warm LMA 0.7%. This cell suspension was placed and distributed on the slide that coated by normal agarose gel. After the polymerization or solidifying of gel on the slide, the slides was put in lysis buffer overnight then they was placed in unwinding buffer for 40 min at 4^{o}C in darkness. The last buffer was used in step of electrophoresis for 20 min at 25 V (1.0 V/cm) and 300 mA. The slides were placed in neutralizing buffer for 5 minutes at 4^{o}C. The head, tail and tail moment were examined under fluorescent microscope by a comet assay device after the staining of slides by ethidium bromide at concentration equals 20 ppm. The tail intensity %, head % and tail moment were obtained by using Comet assay IV software, which was connected to a computer and fluorescent microscope (Perceptive) [14,15].

Statistical Analysis
The results were displayed as mean ±SD. Statistical analysis of data for cytotoxicity and genotoxicity was conducted using one-way analysis of variance (ANOVA) and Duncan's multiple range test (MSTAT-C version 2.1). While the analysis of data of survey was occurred by SPSS version 6.

3. Results and Discussion
Heavy metals released from some types of cookware are affected with temperature, duration and acidity. These metals have many severe health consequence, Awareness concerning choosing good quality of cookware ensure to mitigate diseases related to those toxic metals[16].

Table 1: Correlation coefficient (2 tailed) between awareness, education status and social level

	Awareness	Education status	Social level
Awareness	-	**0.699****	**0.698****
Education status	**0.699****	-	**0.688****
Social level	**0.698****	**0.688****	-

The correlation analysis revealed a statistically significant and strong positive relationship between awareness and both education status (r=0.699**) and social level (r=0.698**) , indicating that individuals with higher educational attainment and higher social standing tend to exhibit greater awareness regarding utensil safety and hygiene . Furthermore, strong correlation was also observed between educational status and social level (r=0.688**) suggesting that these two factors are closely interconnected.
It was revealed that low income in many developing country affect quality of cookware as it is shipper and made from Scrap metal that contaminate food with heavy metals [8]. In other study noted that lead concentrations leached increased in pots made by casting (low cost) than cookware made from rolled aluminium [17] The importance of awareness concerning materials and quality of cookware was recommended by many studies .[6]declared that toxic metals released from various brands of same utensils material was different and that choosing right material and good quality of cooking utensil decrease metals leaching which affect positively health.

Table 2: Correlation coefficient (2 tailed) between awareness, income and each of utensil renouvelation and the use of scratched utensils

	Awareness	Income	Utensil Renouvelation	the use of scratched utensils
Awareness	-	-	-	**-0.376***
Income	-	-	**0.261**	**-0.476****
Utensil renouvelation	-	**0.261**	-	-
The use of scratched utensils	**-0.376***	-	-	-

Awareness showed a moderate positive correlation with both income and utensil renouvelation practices (r=0.261) implying that individuals with higher income levels tend to replace their utensils more regularly and are more aware of potential risks. Importantly, a statistically significant (***p*<0.05**) negative correlation was found between awareness and the used of scratched utensils (r=-0376*) suggesting that as awareness increase, the likelihood of using damaged or scratched utensils decreases. This highlights the importance of educational and income - related factors in influencing safe utensil practices in households.

Heavy metals are considered a major public health problem, migration of such metals from cookware to food severely affect health and can cause various lifestyle diseases. This research studied the amount of some heavy metals leached from old utensils by boiling distilled water under different pH condition for 30 min in them. Results are shown in tables 3 and 4. Cr &Ni were needed with small amount to proper body function, on the other hand exceeding safe limits from these metals was found to be carcinogenic.

Results from these tables revealed that the highest amount of Cr leached was noted in non-stick cookware either in acid or base condition (0.186 and 0.137 mg/L respectively) which represent 4.5 and 2.5
8 times values of control .Followed by ceramic cookware under same pH condition (0.1245 and 0.1142 mg/L) which is equal about 3 and 2.1 times that of acid and base control.

Table 3: Analysis of Chrome and Nickel leaching from cookware under various pH conditions (mg/L)

Element / Material	Chrome			Nickel		
	Acid	**Basic**	**Neutral**	**Acid**	**Basic**	**Neutral**
Pyrex	**0.051**	**0.059**	**0.1032**	**0.0063**	**0.0085**	**0.0073**
Stainless	**0.0756**	**0.075**	**0.1041**	**0.0095**	**0.0105**	**0.0102**
Granite	**0.0956**	**0.0980**	**0.1032**	**0.0111**	**0.0103**	**0.0142**
Non stick	**0.186**	**0.1370**	**0.1037**	**0.0142**	**0.0192**	**0.0141**
Ceramic	**0.1245**	**0.1142**	**0.1037**	**0.0118**	**0.0151**	**0.0145**
Aluminum	**0.089**	**0.088**	**0.1047**	**0.0090**	**0.0102**	**0.0127**
Control	**0.041**	**0.053**	**0.1003**	**0.0055**	**0.0081**	**0.0070**

Nearly same amount of chrome was noted in aluminium and granite cookware under acid condition which represent as much as double that found in control. While stainless had only 84% higher value than that of control.
The amount of chrome leached in neutral solution in non-stick cookware, ceramic and granite cookware arranged in descending order which were 0.093, 0.088 and 0.078 mg /L in. Less amounts of Cr were found in stainless and aluminium. It is worthy to mention that Pyrex had almost equal values of chrome that found in control under same condition.
Nickel is needed in minimal amount for proper body function, while excess amount is very harmful as it was classified on tops of chart causing allergic and different type of cancer such as nose, lung and prostate cancers [18,2] . With regard to the amount of nickel found under acid condition , our results revealed that Ni leached in non-stick, ceramic and granite utensils were higher than those found in other samples and represent about 258 , 214 and 202 % that of control. Same trend was noted under neutral condition. While Ni leached in base solution represent highest amount in non-stick followed by ceramic while each of aluminium, stainless and granite had almost same amount. Our results align with a study carried by [19] ,which demonstrated that stainless may release trace level of chromium and nickel into food during cooking, though within safe limits.

These findings are also consistent with those reported by [3] who observed that even when cooking acidic foods such as tomatoes in stainless cookware the leaching of nickel remained minimal.
Another study noted that cooking acid food was associated with marked increase in leached heavy metals in aluminium cookware [4].
We need to highlight that content of both Ni and Cr in Pyrex are nearly the same as the amount found in the control. It is important to mention that new non-stick cookware coated with Teflon cannot release chrome or nickel, thus we need to ascertain that high amount of leaching such toxic metals found in old non-stick cookware are due to the damage or scratched occurred to the inner coating layer caused by frequent use specially under acidic condition that lead to destroy this layer and

release of metals from base metal of cookware before coating with PTEF[3] . Lead may cause molecular mimicry of divalent metals, thus it may replace some metals (Ca and Zn) in protein and disturb structure and function of protein [2].

Table 4: Leaching of Cadmium and Lead under from cookware at different pH levels (mg/L)

Element / Material	Cadmium			Lead		
	Acid	**Basic**	**Neutral**	**Acid**	**Basic**	**Neutral**
Pyrex	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Stainless	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Granite	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Non stick	**ND**	**ND**	**ND**	**ND**	**0.0101**	**ND**
Ceramic	**ND**	**ND**	**ND**	**ND**	**0.0032**	**ND**
Aluminuim	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Control	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**

*** ND: Not detected =concentration is** below the instrument's detection limit **— the** lowest concentration **that the analytical method can reliably measure (0.0005 – 0.001mg /L for lead and 0.0001 – 0.001mg/L for cadmium) n=2**

The amount of lead and cadmium released from cookware were minimal and often undetectable across different pH . However non-stick and ceramic cookware show low concentration of these metals under basic condition (0.0101 and 0.0032 mg /L respectively). In this concern a study conducted by [2] .Examined leaching elements in rice from different utensils, results of this study revealed that although some metals, leached in tested utensils, exceeded safe limits, old stainless and old aluminium had acceptable values .They also found that low concentration of cadmium was noted in old aluminium and stainless pots.

Another study[20] about cooking meat in different type of utensil, found that cadmium leached was higher in non-stick cookware and aluminium cookware. Migration of heavy toxic metals may also occur from packaging containers [21].

The concentrations of Nickel (Ni) and Chromium (Cr) leached from cookware used in this study (presented in Table 3) were compared with permissible WHO (2022) limits[22] as well as the Egyptian standard (2360/2007 P1 and P2) guidelines for drinking water (0.02 & 0.02 mg/L for nickel and 0.05& 0.054 mg /L for chromium respectively). We could deduce that no sample exceeded limit concerning nickel (0.02 mg/L) while amount leached from non stick under basic condition showed maximum permissible limit value. Although Ni is an essential trace element, elevated levels may cause skin dermatitis, respiratory issues, and nephrotoxicity. It is worst to mention that amount of chromium in both non -stick and ceramic cookware represented about 372 & 274 % (non- stick) and 249 & 228%(ceramic)that of permissible limits under acid and basic condition -in respective order . It was noted that amount of chromium leached in granite and aluminium were about 180% the permissible limits in both acid and basic condition .It is worthy to mention that Pyrex cookware showed least amount leached which are considered closed to control and to the limits mentioned by WHO and Egyptian guideline while stainless exceeded limitation by about 40% . Exposure to Cr can cause gastrointestinal issues, liver damage, and is a known carcinogen. With regard to leached cadmium and lead, only low amount of lead were noted in non- stick (maximum permissible limit)and ceramic cookware under basic condition .

It is important to mention that the amount of heavy metal released from cookware in our study may be due to the scratch occurred in old utensils that was proven to facilitate the leaching of metals.

Heavy metal exposure poses significant health risk to people. These metals can cause various harmful effects, with one of the most concerning being their potential to cause cancer. In our research we examined the amount of metals leached from utensils under different pH conditions, aiming to assess their potential health impacts .

This investigation not only reveals the extent of metal leaching but also emphasizes the importance of evaluating their cytotoxic and genotoxic effects. In the following section will be delved into the cytotoxic and genotoxic evaluation related to the heavy metals identified in our study.

Table 5: Cell viability assessment of cells exposed to leachates from various utensil at alkaline pH

Leaching solution from utensil	Cell viability %
Control	**96.71 ± 1.3^{a}**
Pyrex	**100.00 ±0.0^{a}**
Stainless steel	**93.27 ±1.8^{a}**
Granite	**78.4 ±0.23^{b}**
Tefal	**79.5 ±2.13^{b}**
Ceramic	**98.47 ±2.16^{a}**
Aluminum	**96.65 ±2.98^{a}**

The differences between negative control (represents sodium carbonate solution pH 9) and treatment were occurred by Duncan's multiple range test after one-way ANOVA). The data in the table showed the Mean±SD and the different small letters showed the different significance at $p<0.05$. Sodium carbonate was used as a control

Table 6: Cytotoxic effects of leachate from utensils at acid pH on cell viability

Leaching solution from utensil	Cell viability %
Control	**90.44± 2.50[a]**
Pyrex	**90.38±2.12[a]**
Stainless steel	**80.29±0.15[b]**
Granite	**89.04±0.66[a]**
Tefal	**89.12±2.29[a]**
Ceramic	**89.89±1.13[a]**
Aluminum	**87.00±1.8[a]**

The differences between negative control (represents acid with pH 4) and treatment were occurred by Duncan's multiple range test after one-way ANOVA). The data in the table showed the Mean±SD and the different small letters showed the different significance at $p<0.05$. Diluted acid was used as a control.

Table 7: Impact of neutral pH leachate from different utensils on cell viability

Leaching solution from utensil	Cell viability %
Control	**91.48± 1.8[bcd]**
Pyrex	**97.90±2.12[a]**
Stainless steel	**93.38±2.64[b]**
Granite	**92.05±0.07[bc]**
Tefal	**88.50±1.7[cd]**
Ceramic	**92.40±2.12[bc]**
Aluminum	**87.19±1.48[d]**

The differences between negative control (represents distilled water) and treatment were occurred by Duncan's multiple range test after one-way ANOVA). The data in the table showed the mean ± SD and the different small letters showed the different significance at $p<0.05$. Distilled water was used as control.

Table 8: Genotoxic potential of leachate from Nonstick, Ceramic cookware and control under acidic pH (head% , tail% and tail moment)

Treatment	Head%	Tail%	Tail moment
Control	**72.15±0.07[a]**	**27.85±0.07[c]**	**4.65± 0.1[b]**
Leaching solution of Tefal	**64.26±0.46[b]**	**35.74±0.46[b]**	**5.75± 0.17[b]**
Leaching solution of ceramic	**59.05±0.07[c]**	**40.95±0.07[a]**	**7.99± 1.01[a]**

The differences between negative control (represents diluted acid with pH 4) and treatment were occurred by Duncan's multiple range test after one-way ANOVA). The data in the table showed the mean±SD and the different small letters showed the different significance at $p<0.05$. Diluted acid with pH 4 was used as a negative control.

Table 9: Alkaline pH influence of leachate from Nonstick, Ceramic cookware and control on genotoxicity (head%, tail% and tail moment)

Treatment	Head%	Tail%	Tail moment
Control	**87.96±0.02[a]**	**12.04±0.02[b]**	**1.15±0.05[c]**
Leaching solution of Tefal	**76.84±2.96[b]**	**23.16±2.96[a]**	**3.99±0.04[b]**
Leaching solution of ceramic	**75.33±0.04[b]**	**24.67±0.04[a]**	**4.44±0.02[a]**

The differences between negative control (Alkaline solution with pH 9) and treatment were occurred by Duncan's multiple range test after one-way ANOVA). The data in the table showed the mean±SD and the different small letters showed the different significance at $p<0.05$. Alkaline solution with pH 9 was used as a negative control.

The obtained results serve as a warning about the potential harms that may occur as a consequence of bad utensils materials especially when frequency used and that utensils used either in food factors, restaurants or home may be responsible to the increase prevalence of cancer. We had to mention that while heavy metal leaching (e.g., Ni, Cd, Cr, Pb) is a plausible source of the observed cytotoxic and genotoxic effects in food simulants or cooked food samples, it is important to consider alternative or co-contributing factors, particularly those related to cookware materials and thermal degradation by-products. Some studies suggest that metal ions (e.g., Fe, Cu, Ni) can catalyze the thermal degradation of PTFE, accelerating the formation of reactive fluorinated compounds.
Thus, in a mixed exposure scenario (e.g., from degraded cookware + metal leaching), the combined or synergistic toxic effects may be observed.
While metal migration remains a central concern, non-metallic toxicants such as PTFE degradation products(Carbonyl fluoride, Fluorinated hydrocarbons, etc.)[23].

or fluoride may also contribute to the observed biological effects. Identifying the dominant contributors is essential for accurate risk assessment and consumer safety. Implementing the above experimental approaches will help clarify whether the source of genotoxicity is metals, non-stick coating degradation, or a combination of both.

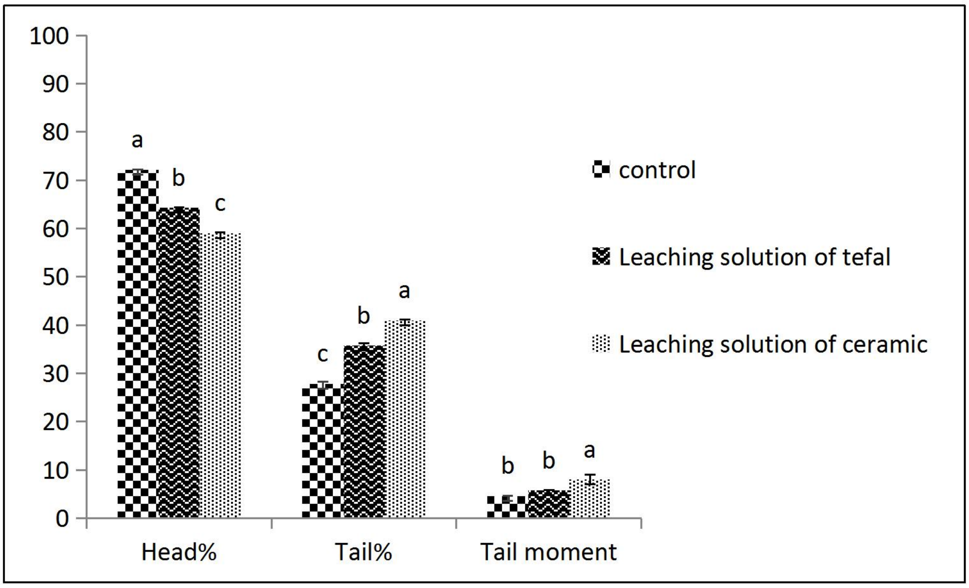

Figure 1: The data of comet assay of the treatment of spleencytes by leaching solutions from utensil under acidic conditions

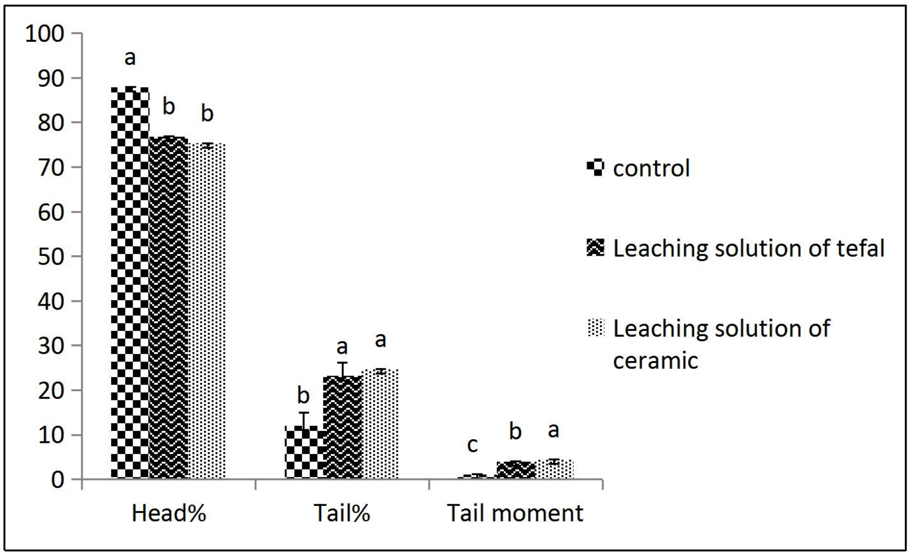

Figure 2: The data of comet assay of the treatment of spleencytes by leaching solutions from utensil under alkaline conditions

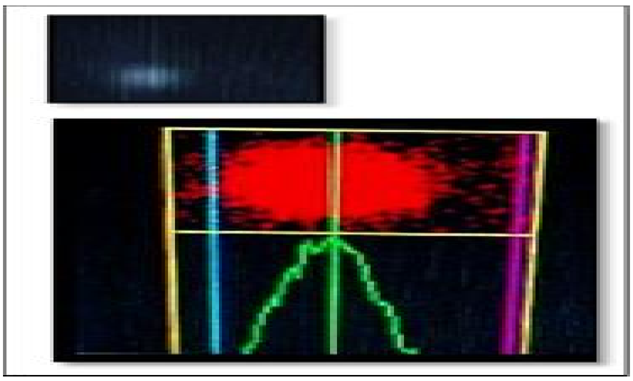

Figure 3: The examination of slide by comet assay device for cells that treated by solution of alkaline leaching (Negative control)

Figure 4: The examination of slide by comet assay device for cells that treated by alkaline leaching solution of non-stick cookware

Recommendation

We emphasize the need for awareness programs to educate people on the importance of selecting appropriate utensils. Furthermore, we recommend that food factories and restaurants ensure procedures about utensils safety and renouvelation.

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