

DISTRIBUTION OF HEAVY METALS IN FLUTED PUMPKIN (TELFEIRIA OCIDENTALIS) LEAVES PLANTED AT DIFFERENT DISTANCES AWAY FROM THE TRAFFIC CONGESTED HIGHWAYS

***Friday E. Uboh¹, Monday I. Akpanabiatu², Emmanuel E. Edet¹ and Iniobong E. Okon³**

¹Department of Biochemistry, Faculty of Basic Medical Sciences,
University of Calabar, Calabar, Nigeria.

²Department of Biochemistry, Faculty of Basic Health Sciences,
University of Uyo, Uyo, Nigeria.

³Department of Botany, Faculty of Sciences,
University of Calabar, Calabar, Nigeria.

***Corresponding author**

ABSTRACT

This study assessed the level of heavy metals (Pb, Ni, Cr, Mn, Cd, and Zn) accumulation in leaves of fluted pumpkin (*Telfeiria occidentalis*) planted at 10, 50 and 100 meters away from three major traffic congested highways, and rural forest farmlands (RFF) in Akwa Ibom and Cross River States in Nigeria. The results obtained from this study showed that the levels of the heavy metals (Pb, Ni, Mn, Cd, and Zn) obtained for the leaves of vegetables planted at 10m from the highways were significantly higher ($P \leq 0.05$) compared respectively to the levels obtained for the leaves at 50 and 100m away from the highways, and the RFF. However, the levels of the heavy metals obtained for the leaves planted within 50m, 100m and RFF were observed to fall within the same concentration range, although the levels for 50m were insignificantly higher ($P \geq 0.05$). This observation indicated that the emissions of heavy metals from the vehicles on traffic congested roads may be deposited on the leaves of vegetables planted at close proximity to the road. Hence, the risk of heavy metals accumulation in leafy vegetables planted within 10m from traffic congested highways is reported in this study.

Keywords : Vegetables, heavy metals, traffic congested highway.

INTRODUCTION

Fluted pumpkin (*Telfeiria occidentalis*) is one of the creeping vegetables that are usually planted on low lands in the tropics. The plant has lobed leaves and long twisting tendrils. Usually, the plant thrives well in soils rich in organic matter and tends to tolerate few meters of elevation above the ground. The vegetable is usually planted in the forest farmlands in the rural areas, as well as along the highways, and gardens around the residential compounds in some rural and urban settlements. Fluted pumpkin is known to be one of the sources of plants' protein, oil, fats, minerals and vitamins in human and animal nutrition [1]. Generally, a variety of vegetable

leaves are used in a balanced diet because they are rich in minerals and vitamins. The leaves of this plant are normally used in some tropical regions, particularly the southern Nigeria, to prepare various delicacies. Specifically, the popular "Edikang Ikong Soup" (a special soup delicacy in Akwa Ibom and Cross River States in Nigeria) is prepared with the pumpkin leaves. It has been reported that the leaves of the pumpkin plants planted in the farmlands, distant from major roads, accumulate no significant amounts of such heavy metals as Pb, Ni, Cd, Zn Mn and Fe [2]. However, the concern of the environmental health scientist is the possible risk of heavy metals bioaccumulation in the leaves of

those vegetables planted along the traffic congested highways. It is well known that high industrial and traffic activities contribute high level of heavy metals to the environments, and plants grown around these areas are likely absorb these metals either from the soil through the roots or from the atmospheric contaminants through the leaves. The heavy metals so accumulated may likely persist and become transferred to those animals, including humans, that feed on them.

Heavy metals are known to be non-biodegradable and persistent environmental contaminants which may be deposited on the surfaces, and then absorbed into the tissues, of vegetables. Plants growing within the heavy metals contaminated areas usually take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils [3-8]. Several reports showed that heavy metals can accumulate in various plant tissues and cause membrane depolarization and acidification of the cytoplasm [9]. Moreover, the report of the study on the phyto-accumulation of some heavy metals in different parts of some cultivated plants species is available [10]. A number of research studies have also shown heavy metals as important contaminants of the vegetables, and that heavy metal contamination of vegetables may also occur due to irrigation with contaminated water [11-15, 6-8]. These reports support the fact that membrane injury is an important effect of metal ions that may lead to cellular disruption of homeostasis.

According to Raje et al. (2005) [16], it has been observed that heavy metals from industry cause serious health problems to human beings. And due to the possibility of bioaccumulation of these metals in plant tissues, and subsequent adverse effect on the health of the consumers, several techniques for the removal of some heavy metals from some medicinal plants have been developed and reported [17-20]. Studies on Cd, Cu and Ni

levels in vegetables from industrial areas of Lagos City, Nigeria carried out by Yusuf et al. (2002) [21] revealed the presence of higher levels of Cd, Cu and Ni in different edible vegetables than those of the residential areas, due to pollution. Also edible portions of five varieties of green vegetables, viz. Amaranth, Chinese Cabbage, Cowpea leaves, Leafy Cabbage and Pumpkin leaves collected from several areas in Dar Es Salaam, Africa, were analyzed Othman et al. (2002) [22] for Pb, Cd, Cr, Zn, Ni and Cu. The result showed a direct positive correlation between Zn and Pb levels in soils with the levels in vegetables. Other studies on the distribution and characterization of heavy metals in vegetable plants and its parts collected from organic farms and village agriculture fields around Anand province, Gujarat, have also been carried out by Nirmal Kumar et al. (2004) [23]. This present work aims to study the distribution of heavy metals in the leaves of fluted pumpkin planted at distances of 10, 50, and 100 meters away from traffic congested highways.

MATERIALS AND METHODS

Sample collection: The fluted pumpkin leaves used in this study were harvested early in the morning (7.00am) from three different farmlands located along three different traffic congested highways linking Akwa Ibom, Cross River and Abia States, and rural forest farmlands in Ikot Akpan Abia – Ubium village in Nsit Ubium Local Government Area of Akwa Ibom State, all in Nigeria. The fluted pumpkin harvested along the highways were planted in farmlands at distances of 10, 50, and 100 meters away from the road, along each of the three highways. At each distance location, in each of the three farm locations, 7 young, tender and fresh leaves were selected and brought to the laboratory for heavy metals assay. Twenty one (21) young, tender and fresh leaves were also harvested from the rural forest farmlands. The leaves harvested from the

farmlands along the highways were placed under running tap to wash off the dirt, and then pulled together into three groups based on the corresponding distance of collection, i.e., Group 1 = leaves harvested at 10 m distance, Group 2 = leaves harvested at 50 m distance, Group 3 = leaves harvested at 100 m distance from each of the highways. While the leaves harvested from the rural forest farmlands were similarly washed to remove the dirt and classified into Group 4. Each group consisted of 21 leaves, which were randomly divided into three subgroups so as to give three determinations per group, during the analysis. The pH of the soil at each sample collection site were measured, and were observed to fall within a close range (6.28 ± 1.02).

Sample preparation: The leaf samples in each of the three groups were air dried to remove the moisture and water droplets. They were then dried to constant weight in an oven maintained at 105°C , and pulverized to fine powder using a laboratory grinder. The ground leaves were collected into well labeled polyethene bags and placed in a desiccator. 3.0g of each sample was carefully weighed into clean platinum crucible and ashed at $450\text{-}500^{\circ}\text{C}$ then cooled to room temperature in a desiccator. The ash was dissolved in 5ml of 20% hydrochloric acid and the solution was carefully transferred into a 100ml volumetric flask. The crucible was well rinsed with distilled water and transferred to the flask and made up to the mark with distilled water and shaken to mix well. The resulting sample solutions were then taken for the determination of the heavy metal concentrations. The samples from each group were analyzed in three determinations.

Sample Analysis: The determination of the heavy metal (Pb, Ni, Cr, Mn, Cd, and Zn) content of the sample solution was carried out using atomic absorption spectrophotometer (Pye Unicam 2900) according to the procedure of the AOAC (1984) on dry samples.

Statistical Analysis: Analysis using one-way analysis of variance (ANOVA) test was carried out to examine the statistical significance of differences in the mean concentration of metals between groups of vegetables using SPSS, version 11. Student's t – test was also used to compared the difference between the mean concentration of each group of the leaf samples. A probability level of $P < 0.05$ was considered statistically significant.

RESULTS

The mean concentrations of heavy metals (Pb, Ni, Cr, Mn, Cd, and Zn) obtained for the pumpkin vegetable leaf samples planted at 10, 50, 100m distances from the traffic congested highways, and rural forest farms are summarized in Table 1. The levels of Pb, Ni, Cr, Mn, Cd, and Zn obtained for the leaves planted at 10m from the highways were 6.32 ± 0.42 , 5.68 ± 0.38 , 1.30 ± 0.08 , 4.50 ± 0.21 , 5.88 ± 0.25 and 4.35 ± 0.16 $\mu\text{g/g}$ respectively, and 1.28 ± 0.03 , 1.65 ± 0.10 , 1.26 ± 0.06 , 1.00 ± 0.05 , 1.70 ± 0.04 and 1.81 ± 0.06 $\mu\text{g/g}$ respectively for leaves planted at 50m from the highways, while 1.11 ± 0.02 , 1.26 ± 0.08 , 1.10 ± 0.08 , 0.96 ± 0.10 , 1.69 ± 0.05 and 1.78 ± 0.06 $\mu\text{g/g}$ respectively, were obtained for leaves planted at 100m from the highways. Also, the levels of the heavy metals obtained for the leaves from the rff were 1.12 ± 0.04 , 1.23 ± 0.08 , 0.98 ± 0.06 , 0.94 ± 0.06 , 1.61 ± 0.05 and 1.80 ± 0.04 $\mu\text{g/g}$ respectively.

These results showed that the levels of Pb, Ni, Mn, Cd and Zn obtained for the leaves harvested at 10m distance from the highways were significantly higher ($P \leq 0.05$) compared respectively to the levels obtained for leaves harvested from the farms at 50, 100m distance from the highways, and rural forest farmlands. However, the levels of these metals obtained for the leaves harvested from the farms at 50, 100m distance from the highways, and rural forest farmlands were not significantly different ($P \geq$

0.05) among and within the groups. From these results, it may be inferred that high levels of Pb, Ni, Mn, Cd and Zn are associated with the vegetable leaves planted at 10m from the highways. The results reported in this study showed that heavy metals accumulation in the vegetable leaves planted along the major highways is dependent on the distance from the road.

DISCUSSION

Vegetables and fruits are generally known to be a good source of vitamins, minerals and fiber, and human beings are usually encouraged to consume them for the benefit of their health. However, these plants, depending on where they are planted and how they are handled after harvest, are known to contain some essential and toxic metals over a wide range of concentrations. This is due to the fact that most plants absorb metals from contaminated soil as well as from deposits on parts of the plants exposed to the air from polluted environments [3, 24]. Food safety is known to be one of the major public concern in almost every society. In the recent past, the increasing demand for food safety has stimulated research on the risk associated with the consumption of food items that have been contaminated by pesticides, heavy metals and toxins [25]. It has been reported that such metals as lead, chromium, cadmium and copper are cumulative poisons, causing various environmental hazards to various organisms (Ellen *et al*, 1990). With the potential toxicity and persistent nature of heavy metals in plant tissues, and the frequent consumption of vegetables and fruits, it has become very important to analyze most of these foodstuffs in order to ensure that the levels of these heavy metal contaminants in plant tissues meet the international requirements [26].

In this study, the levels of Pb, Ni, Mn, Cd and Zn obtained for the vegetable leaves from the planted

close to the road (i. e., 10 meters from the road) were observed to be significantly higher than the levels obtained for the vegetables planted at 50 and 100 meters distances from the highways. Also, the levels of Pb and Cd obtained for the vegetable leaves from the planted at 10 meters distance from the road were observed to be significantly higher than the permissible levels given by the FAO and WHO [27]. However the levels of Pb and Cd, and possibly other metals assessed, obtained for vegetables harvested from plants at 50 and 100 meters from the road were observed be within the permissible levels given by the FAO and WHO. It has been reported that contamination of vegetables with heavy metal may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale [3, 24].

The results of this study therefore support the report that the high heavy metal contamination levels found in some vegetables may be related to pollution from highway traffic [28-29]. The results of this work also agree with the results of the studies carried out by Yusuf *et al.* (2002) [21] on vegetables from industrial areas of Lagos City, Nigeria, Othman *et al.* (2002) on edible portions of five varieties of green vegetables collected from several areas in Dar Es Salaam, Africa, as well as Nirmal Kumar *et al.* (2004) [23] on vegetable plants and its parts collected from organic farms and village agriculture fields around Anand province, Gujarat. Also, Jassir *et al.* (2005) [4] have reported elevated levels of heavy metals in vegetables sold in the markets at Riyadh city in Saudi Arabia due to atmospheric deposition. And recently, Sharma *et al.* (2008a,b) [7-8] reported that the atmospheric depositions can significantly elevate the levels of heavy metals contamination in vegetables commonly sold in the markets of Varanasi, India. These studies revealed the presence of higher levels of

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such metals as Pb, Cd, Cr, Zn, Ni and Cu in different edible vegetables from industrialized areas, due to pollution, than those from the residential areas. The observations made from the results of this study give a strong indication that high traffic congestion introduces heavy metals to the soils and/or atmosphere from where the plants absorb and accumulate them within their tissues.

It has been reported that prolonged consumption of unsafe concentrations of heavy metals through food may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases [30]. Excessive accumulation of such heavy metals as Pb and Cd in food has therefore been reported to be associated with a number of diseases, especially of the cardiovascular, renal, nervous and skeletal systems diseases [30-32, 26]. Some heavy metals such as Cu, Zn, Mn, Co and Mo act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As, and Cr act as carcinogens [33-34], and Hg and Pb are associated with the development of abnormalities in children [35]. Hartwig (1998) [36] and

condition of an area before edible vegetable gardens/farms are sited. From the result of this study, it may be hypothesized that high traffic emissions in traffic congested areas may increase the levels of heavy metals deposition in the environment, leading to significant contamination of vegetables planted within a distance of 10 meters from traffic congested roads. In conclusion, the present study provides data on heavy metal distribution in the pumpkin vegetable leaves planted in farms located at 10, 50 and 100 meters away from traffic congested highways. The levels of heavy metals in the studied vegetables, and the permissible levels required for safe food were compared. High levels of Pb, Ni, Cr, Mn, Cd, and Zn contents were obtained for the vegetables planted at 10 meters from the highways, compared to their permissible levels required for safe food. These amounts may therefore be hazardous to health if large quantities of the vegetables planted within 10 meters distance from the highways are frequently consumed. We therefore suggest that regular monitoring of heavy metals in plant tissues, particularly those planted by the road sides, be carried out in order to prevent excessive accumulation of these metals in the plant tissues,

Group	Pb (µg/g)	Ni (µg/g)	Cr (µg/g)	Mn (µg/g)	Cd (µg/g)	Zn (µg/g)
1	6.32 ± 0.42	5.68 ± 0.38	1.28 ± 0.08	4.50 ± 0.21	5.88 ± 0.25	4.35 ± 0.16
2	1.48 ± 0.03 ^{*,a}	1.65 ± 0.10 ^{*,a}	1.23 ± 0.06 ^{*,a}	1.30 ± 0.05 ^{*,a}	1.86 ± 0.04 ^{*,a}	1.96 ± 0.06 ^{*,a}
3	1.11 ± 0.02 ^{*,a}	1.26 ± 0.08 ^{*,a}	1.18 ± 0.08 ^{*,a}	0.96 ± 0.10 ^{*,a}	1.61 ± 0.07 ^{*,a}	1.78 ± 0.06 ^{*,a}
4	1.12 ± 0.04 [*]	1.23 ± 0.08 [*]	1.18 ± 0.06 [*]	0.94 ± 0.06 [*]	1.61 ± 0.05 [*]	1.80 ± 0.04 [*]

Saplakoglu and Iscan (1997) [37] have reported that long-term intake of Cd caused renal, prostate and ovarian cancers. These heavy metals are also reported to be implicated in carcinogenesis, mutagenesis and teratogenesis [26].

This study therefore reveals that a large daily intake of the vegetables planted within the limit of 10 meters from the highly traffic congested highways is likely to be a health hazard to the consumer(s). Keeping this in mind, it is very necessary to consider the environmental

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and hence the, human food chain.

Table 1. Heavy metals distribution in fluted pumpkin leaves planted at farmlands along traffic congested highways and rural forest farmlands.

Data are presented as mean ± SD of 3 determinations. *P ≤ 0.05 compared to Group 1; ^aP ≥ 0.05 compared to Group 4. (Group 1 = leaves harvested at 10 m distance from each of the highways, Group 2 = leaves harvested at 50 m distance from each of the highways, Group 3 = leaves harvested at 100 m distance from each of the highways, Group 4 = leaves harvested from rural forest farmlands).

REFERENCES

1. Aletor, M.V.A. and O.A. Adeogun, (1995). Nutrients and anti-nutrient composition of some tropical leafy vegetables Food Chem., 53: 375-379.
2. Edem, C. A., Dosunmu, M. I. and Bassey, F. I. (2009). Distribution of Heavy Metals in Leaves, Stems and Roots of Fluted Pumpkin (*Telfeiria occidentalis*). Pakistan Journal of Nutrition 8 (3): 222-224.
3. Khairiah, J., Zalifah, M. K., Yin, Y. H. and Aminha, A. (2004). The uptake of heavy metals by fruit type vegetable grown in selected agricultural areas. *Pak J Biol Sci*; 7: 1438-42.
4. Jassir, M. S., Shaker, A. and Khaliq, M. A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh city, Saudi Arabia. *Bull. Environ. Contam. Toxicol.* 75, 1020-1027.
5. Kachenko, A.G., Singh, B. (2006). Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water Air Soil Pollut.* 169, 101-123.
6. Singh, S. and Kumar, M. (2006). Heavy metal load of soil, water and vegetables in periurban Delhi. *Environ. Monitor. Assess.* 120, 71-79.
7. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008a). Heavy metals (Cu, Cd, Zn and Pb) contamination of vegetables in Urban India: a case Study in Varanasi. *Environ. Poll.* 154, 254-263.
8. Sharma, R. K., Agrawal, M. and Marshall, F.M. (2008b). Atmospheric depositions of heavy metals (Cd, Pb, Zn, and Cu) in Varanasi city, India. *Environ. Monit. Assess.* 142 (1-3), 269-278.
9. Conner, S. D. and Schimid, S. L. (2003). Regulated protal of entry into the cell. *Nature* 422:37 - 44.
10. Neelima, P. and Jaganmohan R. K. (2006). Bioabsorption of some heavy metals in different plant species. *Nature Envir Pollut Tech* 5:53 - 56.
11. Singh, K. P., Mohon, D., Sinha, S. and Dalwani, R. (2004). Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in wastewater disposal area. *Chemosphere* 55, 227-255.
12. Marshall, F. M. (2004). Enhancing food chain integrity: quality assurance mechanism for air pollution impacts on fruits and vegetables systems. Crop Post Harvest Program, Final Technical Report (R7530).
13. Sinha, S., Gupta, A. K., Bhatt, K., Pandey, K., Rai, U.N. and Singh, K.P. (2006). Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physiochemical properties of the soil. *Environ. Monit. Assess.* 115, 1-22.
14. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2006). Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. *Bull. Environ. Contam. Toxicol.* 77, 311-318.
15. Sharma, R. K., Agrawal, M., Marshall, F. M. (2007). Heavy metals contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotox. Environ. Saf.* 66, 258-266.
16. Raje, G. B., Muley, D. V. and Monkar, D. D. (2005). Analysis of heavy metals in ground water from lote industrial area in Ratnagiri District (Maharastra). *J Indust Poll Control* 21:381 - 386.
17. Gupta, S. and Singh, R. P. (2004). Comparative adsorption study of toxic metal by waste product. *Ind J Evt Sci Engg* 24:863 - 866.
18. Feroz, S., King, P. and Prasad, V. S. R. K. (2005). Treatment of metaollic effluent using coconut shell cake. *Ind J Evt Sci Engg* 47:109 - 114.
19. Singh, K. K., Singh, N. L and Hasan, J. H. (2005). Removal of lead from waste water, using agricultural by product wheat bran. *Ind J Evt Prot* 25:390 - 396.
20. Harshad, P., Mhaske Dalvi, K. M. (2006). Determination of some heavy metals from medicinal plant *Pueraria tuberosa* by AAS Tech. *Nat Envir Pollu Tech* 5:389 - 391.
21. Yusuf, A. A., Arowolo, T. O. A. and Bamgbose, O. (2002). Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. *Global Journal of Environmental Science.* 1 (1): 1-6.
22. Othman, O. C. (2001). Heavy metals in green vegetables and soils from vegetable gardens in Dar Es Salaam, Tanzania. *Tanzania Journal of Science.* 27: 37-48.
23. Nirmal Kumar, J. I., Rita N., Kumar, H. S. and Ira B. (2004). Distribution and Characterization of Heavy Metals of Vegetable Plants in and around Anand, Gujarat. Technical Report submitted to Charutar Vidya Mandal (CVM), Vallabh Vidyanagar, Gujarat, India, pp 1- 39.
24. Chojnacka, K., Chojnacki, A., Gorecka, H. and Gorecki H. (2005) Bioavailability of heavy metals from polluted soils to plants. *Sci Total Environ*; 337: 175-182.

<<http://www.sussex.ac.uk/spru/1-4-7-1-11-1.html>>

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25. D'Mello, J. P. F. (2003). Food safety: Contaminants and toxins. Cambridge: CABI Publishing.
 26. Radwan, M. A. and Salama, A. K. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food Chem Toxicol*; 44: 1273-1278.
 27. FAO/WHO. (2001). Food additives and contaminants. Joint Codex Alimentarius Commission, FAO/WHO Food standards Programme, ALINORM 01/12A.
 28. Igwegbe, A. O., Belhaj, H., Hassan, T. M. and Gibali, A. S. (1992). Effect of a highways traffic on the level of lead and cadmium in fruits and vegetables grown along the roadsides. *J Food Safety*; 13: 7-18.
 29. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2009). Heavy metals in vegetables collected from production and market sites of a tropical urban area of India *Food and Chemical Toxicology* 47 : 583–591.
 30. Jarup, L. (2003). Hazards of heavy metal contamination. *Br Med Bull*; 68: 167-182.
 31. WHO. (1995). Lead environmental health criteria. Geneva: World Health Organization, 165.
 32. Steenland, K. and Boffetta, P. (2000). Lead and cancer in humans: where are we now? *Am J Ind Med* ; 38: 295-299.
 33. Feig, D.I., Reid, T.M. and Loeb, L.A. (1994). Reactive oxygen species in tumorigenesis. *Cancer Res.* 54 (Suppl.), 1890–1894.
 34. Trichopoulos, D. (1997). Epidemiology of cancer. In: DeVita, V.T. (Ed.), *Cancer, Principles and Practice of Oncology*. Lippincott Company, Philadelphia, pp. 231–258.
 35. Pilot, C. H., Dragan, P. Y. (1996). Chemical carcinogenesis. In: Casarett, Doulls (Eds.), *Toxicology International Edition*, fifth ed. McGraw Hill, New York, pp. 201–260.
 36. Hartwig, A. (1998). Carcinogenicity of metal compounds: possible role of DNA repair inhibition. *Toxicol. Lett.* 102, 235–239.
 37. Saplakogçlu, U. and Iscan, M. (1997). DNA single-strand breakage in rat lung, liver and kidney after single and combined treatments of nickel and cadmium. *Mutat. Res.* 394 (1), 133–140.
- 38.