

METALS ASSESSMENTS AND HEALTH RISK ASSOCIATED WITH CONSUMPTION OF SOME SELECTED FRUITS OBTAINED FROM ANGWAN RUKUMBA MARKET IN JOS, PLATEAU STATE, NIGERIA.

Abstract.

This work studies the accumulation of selected metals (Mg, Mn, Cr, Cd, Pb, Zn, Fe and Cu) and human health risk via the consumption of selected fruits (Apple, Cucumber, Pawpaw, Sweet Melon and Banana) obtained in Angwan Rukumba Market, Jos Plateau State, Nigeria. The fruits were digested after air-dried with a mixture of HNO₃ and HClO₄ (2;1) and each of these metals were determined using Atomic Absorption Spectrometry (AAS). The results of these metals content in fruit obtained in this market ranged as follows: Apple: Mg (0.630), Mn (0.325), Cr (0.132), Cd (0.174), Pb (0.339), Zn (1.217), Fe (0.374) and Cu (0.218), Cucumber: Mg (0.193), Mn (0.205), Cr (0.102), Cd (0.647), Pb (0.339), Zn (0.231), Fe (0.460) and Cu (0.359), Pawpaw: Mg (0.550), Mn (0.264), Cr (0.701), Cd (0.151), Pb (0.094), Zn (0.086), Fe (0.110), and Cu (0.286), Sweet Melon: Mg (0.164), Mn (0.196), Cr (0.118), Cd (0.108), Pb (0.065), Zn (0.564), Fe (0.285) and Cu (0.246) and Banana: Mg (0.240), Mn (0.187), Cr (0.182), Cd (0.231), Pb (0.363), Zn (0.333), Fe (0.025) and Cu (0.418) in mg/kg respectively, the results of these metals in these fruits are generally lower than WHO/FAO exception of cadmium in cucumber and banana (0.647mg/kg and 0.237mg/kg) which is higher than WHO/FAO values of 0.20mg/kg. The daily intake metal (DIM) values are Mg (0.014 – 0.053), Mn (0.016 – 0.028), Cr (0.009 – 0.060), Cd (0.009 – 0.055), Pb (0.005 – 0.031), Fe (0.002 – 0.040), Zn (0.007 – 0.103) and Cu (0.019 – 0.036) respectively for the five fruits. The Total Hazard Quotient (THQ) values were less than 1.0 for all the metals studied which possibly suggest there is no obvious risk in consuming these fruits from this market.

Keywords: Fruit, Metal, AAS, Aqua-regia, Health.

1.0 INTRODUCTION:

Fruits are of great value and widely used for dietary purposes, important in the diet because of the presence of vitamins and mineral salts. In addition, they contain water, Calcium, Iron, Sulphur and Potassium. They are very important protective food and useful for the maintenance of health and the prevention and treatment of various diseases (Sumainah et al., 2002 and D'Mellow, 2003). However, these plants contain both essential and toxic metals over a wide range of concentrations. Trace metals have been reported to have positive and negative roles in human life because the body need low level of concentration, if this level is exceeded, the effect becomes negative. Some like Cadmium, Lead and Mercury are major contaminants of food supply and may be considered the most important problem to our environment while others like Iron, Zinc and Copper are essential for biochemical reactions in the body.

Generally, most trace metals have long biological half-life and have the potential for accumulation in the different organ of body leading to unwanted side effect (Koleayo et al., 2017). There is a strong link between micronutrient nutrition of plants, animals and humans and the uptake and impact of contaminants in these organisms. The content of essential elements in plants is soil being affected by the characteristics of the soil and the ability of plants to selectively accumulate some metals. Additional sources of Trace metals for plants are: rainfall in atmospheric polluted areas, traffic density, use oil or treatment to plant to **complete** the Maturation of some crops or to give crops attractive look to costumer.

The human body is composed of elements which can be roughly divided into abundant elements and trace elements. Abundant elements consist of the major elements that are involved in the formation of covalent bonds and are important constituents of tissues (oxygen, carbon, hydrogen, nitrogen, etc.), and semi-major elements, which often exist in the ionic state, and are involved in functions of the living body through maintenance of osmotic pressure and membrane potentials (potassium, sodium, etc.). Major elements account for 96% of the total body weight, and the semi-major elements account for 3 to 4% of the total body weight. Deficiency of major elements can lead to nutritional disorders, and their presence in excess can cause obesity. Deficiencies or excess states of semi-major elements often result in water and electrolyte abnormalities.

Essential trace elements of the human body include zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and molybdenum (Mo). Although these elements account for only 0.02% of the total body weight, they play significant roles, for instance, as active centers of enzymes or as trace bioactive substances. A major outcome of trace element deficiencies is reduced activity of the concerned enzymes. However, since each trace element is related to so many enzymes, deficiency of a single trace element is often not associated with any specific clinical manifestations, but rather manifests as a combination of various symptoms.

Because of the presence of trace elements in very small amounts and the absence of specific clinical features associated with their deficiency, it is often difficult for clinicians to identify deficiencies of some particular trace elements. Therefore, this study is aimed at assessing the level of these metals in fruit vegetable obtained from Angwan Rukumba market in Jos, Plateau State and health risk associated with their consumption.

2.0 MATERIALS AND METHODS

2.1 CHEMICALS

All the chemicals used were of analytical grade purchased from Merc Scientific Germany through their office in Lagos, Nigeria.

2.2 SAMPLE COLLECTION AND PREPARATION

Fresh samples of five (5) commonly consumed fruit vegetables (Apple, Banana, Sweet Melon, Cucumber and Pawpaw), were obtained from Angwan Rukumba Market in Jos North Local Government Area of Plateau State, Nigeria. The samples were properly labeled in a polythene bag and taken to the herbarium of Plant Science and Technology Department of University of Jos for identification, and subsequently taken to the Postgraduate Chemistry laboratory University of Jos, where it was washed with tap water thoroughly to remove all debris, dust particles, soil and rinsed with cleaned water severally. These washed fruits vegetables were air dried in the laboratory at room temperature for three days after which they were chopped into small pieces and oven dried at 55°C then crushed into fine powder using a porcelain mortar and pestle and kept in air tight bottle. 0.5 g of each sample was measured into a clean dried beaker (200 cm³), 10cm³ of acid mixture of HNO₃/HClO₄ in a ratio 2:1 was added to the sample digestion, it was allowed to be distributed evenly in the acidic medium with constant stirring with a glass rod, this was then placed on the digestion block in a fume cupboard for 2 hours at the temperature of 150°C for complete digestion. The digested samples were then filtered into 50 cm³ volumetric flask and made to mark with distilled water, this was run in AAS Buck Scientific model 210 VGP after

running a set of standards to obtain a calibration curve and the results of each metal of interest was extrapolated.

2.3 HEALTH RISK ASSESSMENT:

The health risk assessment of metal consumption via fruit vegetables were assessed based on the daily intake of metal (DIM), health risk index (HRI) and target hazard quotient (THQ) (Chary et al., 2008, Jan et al., 2010, Storelli, 2008 and Wang et al., 2005). The daily intake of the metal (DIM) was calculated to estimate the average daily metal loading into the body system of a specified body weight of a consumer, this was calculated by the formula

$$DIM = \frac{C_{\text{metal}} \times C_{\text{factor}} \times C_{\text{foodintake}}}{\text{Average body weight}} \quad \text{-----} \quad (1)$$

Where C_{metal} is the metal concentration in vegetable (mg/kg), C_{factor} is the conversion factor, $C_{\text{foodintake}}$ is the daily intake of vegetables. The conversion factor of 0.085 is to convert fresh vegetable weight to dry weight (Sajjad et al, 2009), daily intake of 65g/day (Oguntona, 1998) while the average body weight used was 65 kg for this study (Oguntona, 1998)

The health risk index (HRI) was calculated using the formula:

$$HRI = \frac{DIM}{RFD} \quad \text{-----} \quad (2)$$

HRI = Health risk index, DIM = daily intake of metal and RFD = oral reference dose of metal

The THQ was calculated using the formula:

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA} \times 10^{-3} \quad \text{-----} \quad (3)$$

Where EF is the exposure frequency (350 days/year), ED is the exposure duration (54years equivalent to the average lifetime of the Nigeria population), FIR is the food ingestion rate (vegetable consumption values for adult Nigeria is 65 g/person/day) (Oguntona, 1998), C is the metal concentration in the vegetable mg/kg, RFD is the oral reference dose (Pb, Cd, Cu, Zn, Cr, Mn and Fe)(0.0035, 0.001, 0.040, 0.03, 1.50 and 0.007), WAB = average body weight (65kg), TA = Average exposure time for non-carcinogens (ED x 365 days/year). If the THQ value is greater than 1, the exposure is likely to cause obvious adverse effects. (Adedokun et al., 2016)

3.0 RESULTS AND DISCUSSION

The results of selected metals in the fruits investigated were presented in table 1, daily intake rate in table 2 and health risk index and target hazard quotient in table 3 respectively.

Table 1 Metal concentrations in some selected Fruits sold in Angwan Rukumba Jos, Plateau State, Nigeria

Element/Sample	Apple	Cucumber	Pawpaw	Sweet Melon	Banana
Mg	0.630 ± 0.056	0.193 ± 0.031	0.550 ± 0.052	0.164 ± 0.023	0.240 ± 0.016
Mn	0.325 ± 0.056	0.205 ± 0.035	0.264 ± 0.046	0.196 ± 0.034	0.187 ± 0.032
Cr	0.132 ± 0.023	0.102 ± 0.013	0.701 ± 0.035	0.118 ± 0.020	0.182 ± 0.032
Cd	0.174 ± 0.030	0.647 ± 0.112	0.151 ± 0.026	0.108 ± 0.019	0.231 ± 0.040
Pb	0.202 ± 0.035	0.339 ± 0.059	0.094 ± 0.016	0.065 ± 0.007	0.363 ± 0.053
Zn	1.217 ± 0.211	0.231 ± 0.040	0.086 ± 0.008	0.564 ± 0.048	0.333 ± 0.032
Fe	0.374 ± 0.065	0.460 ± 0.038	0.110 ± 0.019	0.285 ± 0.045	0.025 ± 0.004
Cu	0.218 ± 0.038	0.359 ± 0.052	0.286 ± 0.049	0.246 ± 0.043	0.418 ± 0.052

Table 2 Daily intake rate (mg person⁻¹ day⁻¹) of metal in fruits obtained from Angwan Rukumba Market, Jos Plateau State, Nigeria

Fruits	Mg	Mn	Cr	Cd	Pb	Zn	Fe	Cu
Apple	0.053	0.028	0.011	0.015	0.017	0.103	0.032	0.019
Cucumber	0.016	0.017	0.009	0.055	0.029	0.020	0.040	0.031
Pawpaw	0.047	0.022	0.060	0.013	0.007	0.007	0.009	0.024
Sweet Melon	0.014	0.017	0.010	0.009	0.005	0.048	0.024	0.020
Banana	0.020	0.016	0.015	0.020	0.031	0.028	0.002	0.036

Table 3 Health risk index (HRI) and target hazard quotient (THQ) for metals in Selected Fruits obtained in Angwan Rukumba Market, Jos Plateau State, Nigeria

Fruits		Apple	Cucumber	Pawpaw	Sweet Melon	Banana
Mg	HRI	-	-	-	-	-
	THQ	-	-	-	-	-
Mn	HRI	2.000	1.214	1.571	1.214	1.143
	THQ	0.023	0.015	0.019	0.014	0.013
Cr	HRI	0.229	0.188	1.250	0.208	0.313
	THQ	0.003	0.002	0.015	0.002	0.004
Cd	HRI	15.000	55.000	13.000	9.000	20.000
	THQ	0.174	0.647	0.453	0.108	0.231
Pb	HRI	4.857	8.286	2.000	1.429	8.857
	THQ	0.058	0.097	0.027	0.019	0.104
Zn	HRI	0.343	0.067	0.023	0.160	0.093
	THQ	0.004	0.000	0.000	0.002	0.001
Fe	HRI	0.046	0.057	0.013	0.034	0.029
	THQ	0.000	0.000	0.000	0.000	0.000
Cu	HRI	0.475	0.775	0.600	0.500	0.900
	THQ	0.005	0.010	0.007	0.006	0.010

The results of metals in various fruits vegetable studied indicated in table 1 revealed Zinc had the highest concentration in Apple with the concentration of 1.217 mg/kg, Zinc is essential in human diet and is required to maintain the functioning of the immune system. It is also a natural constituent of soil in terrestrial ecosystem and it is taken up actively by roots (Adesuyi, et al., 2015). After Zinc followed by Magnesium with the concentration of 0.630 mg/kg and least is Chromium with the concentration of 0.132 mg/kg, Chromium depending on the valent state, can

be beneficial or harmful (Adedokun, 2016). Chromium is known to help maintain normal blood glucose levels by enhancing the effects of Insulin (Chove et al., 2006). The effect of Chromium allergy caused by exposure to Chromium especially Cr^{+6} assumed to be a carcinogenic agent (RTI, 2000) The concentrations of these metals are in the order of $\text{Zn} > \text{Mg} > \text{Fe} > \text{Mn} > \text{Cu} > \text{Pb} > \text{Cd} > \text{Cr}$. The concentrations of these metals in apple were compared with the guideline values of the FAO/WHO (2001), it was discovered all the values were within the recommended values which means eating Apple from this market with these concentrations of metals could possibly enrich the health status of the consumers.

For Cucumber the results revealed Cadmium had the highest concentration with the value of 0.647 mg/kg, cadmium is a heavy metal with high toxicity and it is a non-essential element in foods and natural waters and it accumulates principally in the kidneys and liver (Divrikli, et al., 2006, Adesuyi, et al., 2015). Higher values have been previously reported for leafy vegetables cultivated along roadsides to be 0.27 mg/kg by Oluwole et al, (2013), major sources of cadmium in plants and fruit vegetables sewage sludge application, deposition from fossil fuel combustion, phosphate fertilizer etc. (Adesuyi et al., 2015). Cadmium accumulates especially in the kidney with increased secretion of proteins in urine and other effects (Waalkes, 2000), this concentration in Cucumber followed by Iron with 0.460 mg/kg and least with Chromium with 0.102 mg/kg respectively. The order followed the pattern $\text{Cd} > \text{Fe} > \text{Cu} > \text{Pb} > \text{Zn} > \text{Mn} > \text{Mg} > \text{Cr}$. The concentration of Cadmium in Cucumber (0.647 mg/kg) and Lead (0.339 mg/kg) are higher than the guideline values of 0.20 and 0.3 mg/kg set by FAO/WHO (2001), this calls for caution. The higher concentrations of Cadmium and Lead could possibly be attributed to the soil, water or possible vehicular emission since the market is located by one of the major roadsides in Jos where traffic is high. All other metals studied in Cucumber were within the recommended values. Meanwhile for Pawpaw all the metals investigated were within the guideline limit set by FAO/WHO (2001), with Chromium (0.701 mg/kg) followed by Magnesium (0.550 mg/kg) and least with Zinc (0.086

mg/kg). The order followed Cr > Mg > Cu > Mn > Cd > Fe > Pb > Zn. However, all the metals studied in Sweet Melon were below the guideline values set by WHO/FAO with Zinc having the highest concentration 0.564 mg/kg followed by Iron with 0.285 mg/kg and least with Lead 0.065 mg/kg, the overall pattern of metals investigated in Sweet Melon are Zn > Fe > Cu > Mn > Mg > Cr > Cd > Pb. Nevertheless, for Banana the results of Cadmium 0.231 mg/kg and Lead 0.363 mg/kg were higher than the recommended values of 0.20 and 0.3 mg/kg set by FAO/WHO (2001), all other metals had concentrations within the guideline value with copper having the highest (0.418 mg/kg), copper is essential to human life as metalloproteins and function as enzymes, however critical doses lead to health risks such as anemia, diabetes, inflammation, kidney and liver dysfunction and vitamin C deficiency (Lokeshappa et al., 2012) and least with Iron (0.025 mg/kg). The pattern in Banana followed Cu > Pb > Zn > Mg > Cd > Mn > Cr > Fe. Lead pollution has been attributed to population/vehicular density (Afolami et al., 2010). Generally, lead contaminations occur in vegetables grown on contaminated soils, through air deposition or through sewage sludge, waste water application (Oluwole et al., 2013). Lead poisoning is a world-wide reality, but fortunately is not a very common clinical diagnosis yet in Nigeria except for few occupational exposures (Anetor et al., 2008). The results obtained here is similar to that reported by Asdeo and Loonker (2011) and Adedokun et al (2016) who reported the values of Cadmium and Lead higher than the permissible limit for vegetables. Lead is causing concern in particular due to the possible impacts on children, Lead influences the nervous system, slowing down nervous response, this influences learning abilities and behaviour (Adesuyi et al., 2015).

For the daily intake rate indicated in Table 2, the results of magnesium in the five fruit vegetables sample studied was from 0.014 – 0.053, manganese was from 0.016 – 0.028, chromium was 0.009 – 0.060, cadmium was from 0.009 – 0.055, lead was from 0.005 – 0.031, zinc was from 0.007 – 0.103, iron was from 0.002 – 0.040 and copper was from 0.019 – 0.036. All these values were all

within the FAO/WHO (2001) recommended values for the metals investigated, none of these metals had value above the guideline limit only that most were far below the limit. The daily intake of metals is useful in establishing health risk associated with any pollutants by estimate the level of exposure according to the average fruit vegetable consumption.

Table 3 indicated the results of health risk index and target hazard quotient. From the results it can be seen that the target hazard quotient of all the metals studied are less than one meaning there could not be any obvious risk in consuming these fruits. The HRI for all the fruit vegetables ranges as follows Mn (1.143 – 2.00), Cr (0.188 – 1.250), Cd (9.000 – 55.000), Pb (1.429 – 8.857), Zn (0.023 – 0.343), Fe (0.013 – 0.057) and Cu (0.475 – 0.900). The HRI for Mn, Cr, Cd and Pb from the study were greater than 1 ($HRI > 1$) while HRI for Zn, Fe and Cu are less than 1 ($HRI < 1$). Generally, means that the exposed population $HRI < 1$ is safe of metal risk while $HRI > 1$ means the reverse (khan et al, 2008). The population is therefore at greater risk of Cd, Pb and Mn.

CONCLUSION

With increasing health awareness and the growing number of fruits nowadays, fruits safety is a very important issue. The present study has generated data on metals contamination in fruit vegetables sold in Angwan Rukumba market, Jos, Plateau State, Nigeria and associated risk assessment for consumer's exposure to these metals. The levels of eight metals (Mg, Mn, Cr, Cd, Pb, Zn, Fe & Cu) were all within the guideline limit of FAO/WHO in apple, pawpaw, sweet melon, cucumber and banana exception of Cadmium value for cucumber (0.647 mg/kg), Banana (0.237 mg/kg) were above the permissible limit of 0.20 mg/kg and lead in cucumber (0.339) and banana (0.363) in mg/kg were also above the permissible limit of 0.30 mg/kg. The daily intake of these metals Mg, Mn, Cr, Cd, Pb, Zn, Fe & Cu are between 0.002 – 0.103 all less than 1.0. It can be suggested that the consumption of average amount of these contaminated fruit vegetables does not pose a health risk for consumers as the values obtained are below the FAO/WHO limits for metals intake, but certain groups of consumers such as elderly with cardiovascular problems and

kidney deficiency who intake of cucumber and banana in this market for long term should be extra cautions as they are more susceptible to toxicities. Hence it is suggested that regular monitoring of metals in fruit vegetables and other food items should be carried out to prevent excessive buildup of these metals in the human food chain. Care should also be taken at the time of production, transportation and marketing of fruit vegetables.

REFERENCES

Adedokun, A. H., Njoku, K. L., Akinola, M. O., Adesuyi, A. A and Jolaoso, A. O (2016) Potential Human Health Risk Assessment of Heavy Metals intake via consumption of some leafy vegetables obtained from four market in Lagos Metropolis, Nigeria. *Journal of Applied Science and Environmental Management* 20(3) 530- 539.

Adesuyi, A. A., Njoku, K. L and Akinola, M. O (2015) Assessment of heavy metals pollution in soils and vegetation around selected industries in Lagos state, Nigeria. *Journal of Geoscience and Environmental protection* 3: 11-19.

Afolami, L., Anyakora, C., Ebuchi, O and Bolawa, O (2010) Lead levels in some edible vegetables in Lagos, Nigeria. *Scientific Research and Essays* 5(8) 813- 818.

Anetor, J. T., Anetor, G. O., Udah, D. C and Adeniyi, F. A (2008) Chemical carcinogenesis and chemoprevention: Scientific priority area in rapidly industrializing developing countries. *Africa Journal of Environmental Science and Technology* 2(7) 150-156.

Asdeo, A and Loonker, S (2011) A comparative analysis of trace metals in vegetables. *Research Journal of Environmental toxicology* 5: 125 – 132.

Chery, N. S., Kamala, C. T and Raj, D. S. S (2008) Assessing Risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental safety* 69: 513 – 524.

Chove, B. E, Ballegu, W. R and Chove, L. M (2006) Copper and Lead levels in two popular leafy vegetables grown around Morogoro Municipality, Tanzania. *Tanzania Health Bulletin* 8(1) 37 – 40.

D'Mellon, J.P.F (2003) Food Safety, contamination and Toxicants, CABI Publishing Wallingford, Oxon UK, Cambridge, MA, P48

Divrinkli, U., Horzum, N., Soyak, M. and Eki, L (2006) Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. *International Journal of food science and technology* 41: 712 – 716.

FAO/WHO (2001) Report on the 32nd session of the codex committee on food additives and contaminants ALINORM 01/12 Beijing China 20 – 24 March 2000 Joint FAO/WHO food standard programme: Codex Alimentarius commission 24th session 2 – 7 July Geneva Switzerland.

Jan, F. A., Ishaq, M., Khan, S., Ihsanullah, I., Ahmed, I and Shakirullah, M (2010) A comparative study of human health risk via consumption of food crops grown on waste water irrigated soil (Peshawar) and relatively clean water irrigated soil (Lower Dir) *Journal of hazard materials* 179: 612 – 621.

Khan, S., Lin, A., Zhang, S., Huc, Q and Zhu, Y (2008) Accumulation of polycyclic aromatic hydrocarbon and heavy metals in Lettuce grown in the soils contaminated with long term waste water irrigation. *Journal of hazardous material* 152: 506 – 515.

Koleayo, O. O., Kelechi, L. N., Olutunde, O. B and Olapeju, A. A (2017) Nutritional Composition and heavy metal content of selected fruits in Nigeria. *Journal of Agriculture and Environment for International Development* 111(1) 123 - 135

Lokeshappa, B., Shivpuri, K, Tripathi, V and Dikshit, A.K (2012) Assessment of toxic metals in Agricultural produce food and public health 2(1) 24 – 29.

Oguntona, T (1998) Green leafy vegetables. In: Osagie, A. U and Eka, O. U (Eds) National quality of plant foods post-harvest research unit Department of Biochemistry University of Benin. Benin City, Nigeria pp 120 – 133 IBSN 978 – 2120 -02 – 2

Oluwole, S. O., Makinde, O. S. L., Yusuf, K. A., Fajana, O. O and Odumosu, A. O (2013) Determination of heavy metal contaminants in leafy vegetables cultivated by the road side. *International Journal of Engineering Research and Development* 7(3) 1-5.

RTI (2000) Toxicological profile for chromium Syracuse research corporation for US Department of Health and Human services, Agency for toxic substances and diseases registry, Atlanta.

Sajjad, K., Robina, F., Shagufta, S., Mohammad, A. K and Maria, S (2009) Health Risk assessment of Heavy Metals for Population via consumption of vegetables. *World Applied Sciences Journal* 6(12) 1602 – 1606.

Storelli, M. M (2008) Potential human health risks from metals (Hg, Cd and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target quotients (THQs) and toxic equivalents (TEQs) *Food Chemistry and Toxicology* 46: 2782 – 2788.

Sumainah, G and Al-jebahM, (2002) The level of trace metals in vegetable crops collected along the irrigation water source of Barada – AL- Ghouta Damascus University Magazine of basic science (18) NO 2,2001. published 2002.

Waalkes, M. P (2000) Cadmium carcinogenesis in review *Journal of inorganic Biochemistry* 79: 240 – 244.

Wang, X., Sato, T., Xing, B. and Tao, S (2005) Health Risks of heavy metals to the general public in Tianjin China via Consumption of vegetables and fish. *Science and total environment* 350: 28 – 37.

UNDER PEER REVIEW