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The abstracts have been arranged in alphabetical order using the surname or family name of the presenting author. The family or surnames of the authors have been underlined and the names of the presenting authors are in bold.

**TREND MONITORING OF FLUORIDE IN GROUNDWATER RESOURCES OF IRAN  
FROM 2003 TO 2010 USING THE MANN-KENDALL TREND TEST AND THE  
GEOSPATIAL INFORMATION SYSTEM (GIS)**

Spatio-temporal variations of fluoride (F) from 2003 to 2010 in groundwater of the Larestan and Gerash regions in Iran were monitored employing the Mann-Kendall trend test and a geospatial information system (GIS). The annual precipitation had a negative but not significant relation to the mean concentrations of F in the wells of eight cities (Pearson  $r = -0.512$ ,  $p = 0.195$ ) and thirty-one villages ( $r = -0.545$ ,  $p = 0.259$ ) for the eight-year period. Results of the Mann-Kendall trend test revealed a trend in the series of 1 city and 11 village wells for the eight-year period (significant level  $\alpha = 0.05$ ). Spatio-temporal variations of groundwater F were relatively constant over the years. In 2003 to 2010, 62.8, 63.2, 63.4, 62.8, 62.4, 62.7, 58.9, and 59.5 percent of the Larestan and Gerash region areas had groundwater F concentrations above the maximum permissible standard F level of 1.4 mg/L. Accordingly, effective protective measures, cost-effective interventions, and compliance monitoring—even monthly monitoring—should be considered, especially in unsafe contaminated areas to achieve a standard level for health safety.

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Keywords: Environmental contamination; Geospatial information system (GIS); Groundwater; Health; Iran; Mann-Kendall trend test; Spatio-temporal variations.

**FLUORIDE REMOVAL USING AGRICULTURAL WASTE RICE HUSK AS A  
LOW-COST ADSORBENT**

Among many public health officials, fluoride is considered a vital constituent for humans; it can be either useful or harmful to human health, depending on its level in drinking water. Fluoride concentration levels of 1–1.5 mg/L are widely regarded as beneficial for teeth, but concentrations in the range of 1.5–4 mg/L and 4–10 mg/L

L result in dental fluorosis and skeletal fluorosis, respectively. In this study, the removal of fluoride by rice husk and rice husk carbon was studied by batch technique to obtain equilibrium data and the rate of adsorption. Effects of various parameters such as temperature, pH, adsorbent dose, concentration of fluoride, agitation time, and rate of adsorption were investigated. The results showed that pH has a significant effect on fluoride adsorption. With increasing adsorbent dose, fluoride removal increased. Agitation rate and agitation time were also important variables in this study. Fluoride removal efficiency also decreased with increase in initial fluoride concentration under constant conditions.

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Keywords: Activated carbon; Adsorption; Fluoride removal; Rice husk; Water defluoridation.

## ENDEMIC FLUORIDE POLLUTION IN DRINKING WATER AND ITS IMPACT ON HUMAN HEALTH AND MANAGEMENT BY BIO-REMEDICATION

Fluoride is a highly electronegative element naturally present in the earth's crust as well as in rocks and minerals. Exposure to fluoride-contaminated drinking water beyond the recommended level for long periods of time causes fluorosis—an irreversible crippling disease that may lead to serious public health problems. The present study was undertaken to determine fluoride levels in drinking water of 19 blocks of the Birbhum district, West Bengal. The fluoride concentration in ground water of some villages was found to exceed the maximum permissible limit, e.g., in Nasipur with 46 mg F/L (almost 40 times higher than the WHO recommended limit). In these areas, villagers are afflicted with dental, skeletal, and gastro-intestinal fluorosis. From our nutritional surveys of people in the fluoride-affected areas and statistical analysis (with the help of S+ software), we have found that a poor nutritional level enhances the fluorosis problem. We have also isolated three bacterial strains from fluoride-contaminated wells. These bacteria have the ability to survive in a medium containing high concentrations of fluoride ions. A simplified mathematical model of fluoride and bacterial dynamics is proposed. Analytical solutions show that fluoride uptake and bacterial growth rates are the most important parameters and asymptotic stability of the system. We have also found that some fluoride-accumulating plant species reduce the fluoride content of soil and water. The present work is focused on the actual need of nutrients for people to resist fluorosis in the contaminated area and on the potentiality of biotechnology to remove fluoride from contaminated water and soil with the help of bacteria and plant species.

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Keywords: Asymptotic stability; Bacteria; Bio-remediation; Fluoride accumulating plants; Fluorosis; Mathematical model; Nutritional survey.

### **FLUORIDE CONCENTRATION IN DRINKING WATER SUPPLIES IN QOM, IRAN, AND DMFT INDEX OF 12-YEAR-OLD STUDENTS**

The fluoride concentration in various drinking water resources of Qom province, Iran, and its relationship with the DMFT dental index of 12-year-old students was investigated. A total 270 samples were collected from different drinking water sources, which consist of drinking water systems relying on private, public and household desalination units. A total of 480 students 12 years of age were randomly selected from 24 primary schools with 20 students from each school. The entering criterion for each participant selected was at least 3 years of continuous consumption of each water source. Average fluoride concentrations in the water from the public saline distribution system (PSDS), the public desalinated distribution system (PDDS), private desalinated water suppliers (PDWS), and household desalinated water units (HDWU) were 0.45 mg/L, 0.16 mg/L, 0.1 mg/L, and 0.18 mg/L, respectively. The DMFT index of populations consuming water from PSDS, PDDS, PDWS, and HDWU was 3.1, 2.44, 2.8, and 1.99, respectively. Results reveal a direct relationship between fluoride concentrations of some resources (PSDS and HDWU) and DMFT index. Interestingly, the highest fluoride concentration (0.45 ppm) is associated with the highest DMFT (3.1), which is close to the DMFT (2.8) of the lowest fluoride concentration (0.1 ppm). It seems, therefore, that factors other than fluoride levels in the water, such as mouth rinse use, parent educational level, and tooth brushing frequency, have more important effects on the DMFT index. There were no obvious differences between DMFT among populations satisfied with PDDS and PDWS water. Consideration, however, should be given to water desalination systems during installation, operation, and maintenance regarding provision for by-pass lines to compensate for differences in desired fluoride concentrations. Because of the important effects of other factors besides fluoride, emphasis is recommended for parental educational programs regarding dental hygiene of their children.

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Keywords: Desalination; DMFT index; Drinking water; Qom, Iran; Student dental survey.

### **EFFECT OF FLUORIDE STRESS ON PHOTOSYNTHETIC PERFORMANCE OF THREE SEMI-ARID PLANT SPECIES**

At lower ambient fluoride (F) concentrations, a number of physiological and biochemical changes may be initiated in plants without the appearance of visible symptoms of fluoride toxicity. Three tree species in the semi-arid region of Pauri, India, viz., *Acacia tortilis*, *Cassia fistula*, and *Prosopis juliflora*, grow very well in F-contaminated areas. To detect changes in photosynthesis due to F stress, the chlorophyll content (chlorophyll a, chlorophyll b, and the chlorophyll a/b ratio) and chlorophyll fluorescence measurements were carried out in one-month-old plants treated with 10, 20, and 50 mg F/kg for 10 days. Visible injury in the form of chlorosis was observed only in leaves of *C. fistula*, which seemed to be more sensitive to F stress, since maximum decrease in chlorophyll content was also observed in this plant. Short-term exposure to F did not cause any major change in the photosynthetic performance of these plants. This was revealed by ETR, ETR<sub>max</sub>, PPFD-sat'd, and *f*F/F<sub>m</sub>-sat'd values, which were higher in these plants. Thus, F treatment did not cause any deleterious effects on chlorophyll content and photosynthetic performance in these plants during early growth stages. The lesser inhibition in the parameters confirms the F-tolerant nature of these tree species, thereby indicating their potential use for treatment of F-contaminated soil and water.

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### **FLUORIDE REMOVAL BY AN ELECTRO-COAGULATION USING IRON AND ALUMINUM ELECTRODES**

Fluoride in drinking water above permissible levels is often responsible for skeletal fluorosis in humans. The present study was carried out to assess the ability of electro-coagulation with iron and aluminum electrodes to remove fluoride from aqueous solutions. Several working parameters, such as fluoride concentration, pH, applied voltage, and reaction time, were studied to achieve a higher removal capacity. Variable concentrations (1, 5, and 10 mg/L) of fluoride solutions were prepared by mixing proper amounts of sodium fluoride with deionized water. Varying the pH of the initial solution (3, 7, and 10) was also studied to measure effects on the fluoride removal efficiency. Results with synthetic solutions revealed that the most effective fluoride removal capacity could be achieved at an electrical potential of 40 V. In addition, increase of electrical potential in the range of 10–40 V enhanced the rate of fluoride removal. Also, comparison of fluoride removal efficiency showed it to be similar with iron and aluminum electrodes. Finally, it can be concluded that electro-coagulation has potential for cost-effective removal of fluoride from water and wastewater.

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Keywords: Aqueous environment; Electrocoagulation; Fluoride removal.

### **MORPHOLOGICAL PICTURE OF THE KIDNEYS AND THE ACTIVITY OF SELECTED ENZYMES AFTER ADMINISTRATION OF VITAMIN E AND METHIONINE TO RATS EXPOSED TO SODIUM FLUORIDE**

In doses exceeding the safe-dose limit, fluoride ions have multidirectional toxic effects on living organisms. They induce pathomorphological changes in the parenchymal organs and affect the metabolic pathways of lipids, proteins, and carbohydrates. DNA damage is also observed. Recent studies have shown that fluoride plays an important role in causing pathological changes and disorder in oxidation-reduction processes. Our experiment was conducted on 18 Wistar strain male rats with average body mass before the experiment of  $393.39 \pm 47.54$  g. The rats were divided into three groups with 6 animals in each group. Rats in the control group were given distilled water. The animals in both experimental group I and group II received 10 mg NaF/kg of body mass/24 hr in water solution. In addition, a second experimental group of rats was administered methionine (2 mg/rat/24 hr) and vitamin E (3 mg/rat/24 h) along with NaF. The experiment lasted 35 days. After this time the animals were weighed, then put to death by intraperitoneal administration of hexobarbital. Upon dissection, the kidneys of the rats were removed and fixed in formalin. Slide preparations were made by the paraffin method and stained with hematoxylin and eosin. In the supernatants obtained after homogenization of the kidneys in saline and centrifugation, the enzyme activities of carbohydrate metabolism: fructose-1,6-bisphosphate aldolase (ALD), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH) and glutathione-S-transferase (GST), glutathione reductase (GR), and glutathione peroxydase (GPx) were measured. Results showed an adverse effect of fluoride on kidney histopathology. In evidence were edema, disseminated necrosis of renal tubular epithelial cells, and hyperaemia of nutrient capillaries of the renal tubules. In addition, there were narrowing Bowman spaces and hyperaemia of the glomerular capillaries. Furthermore, small infiltration of mononuclear cells into the stroma was also observed. In addition, exposure to NaF caused a decrease in the activity of enzymes involved in carbohydrate metabolism. This adverse effect was associated with a statistically significant decrease in activity of malate dehydrogenase (MDH), indicating alterations in energy processes of the cell. Interference in the Krebs cycle was also observed, with slight reduction in the activity of enzymes associated with glutathione. Finally, combined administration of antioxidants showed beneficial effects on the activity of enzymes in the kidney of rats exposed to sodium fluoride and inhibited the pathomorphological alterations that developed in the kidneys following sodium fluoride intoxication.

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### **FLUORIDE LEVELS IN URBAN WATER DISTRIBUTION NETWORK IN THE CITIES OF SARI, NOSHAHR, TONEKABON, AND RAMSAR IN IRAN DURING 2011–2012**

Fluoride occurs naturally in low concentration in food and water. In 1931, fluoride was discovered to be the cause of human enamel mottling known as dental fluorosis. The major sources of exposure to fluoride for people are drinking water and food. The current study was undertaken to evaluate the fluoride level in the urban water distribution networks of the cities of Sari, Noshahr, Tonekabon, and Ramsar in Iran. The results have been compared to standards for universal national, and climatic conditions. This descriptive study is sectional, according to the number of water sources and by urban water and wastewater department consultation. Sampling was done in September and October of 2011. The samples were taken to the Karaj Applied Geology and Research Center, where the fluoride concentration was measured by the 9056A EPA method. The results showed that the mean maximum and the minimum level of fluoride in drinking water is 0.289 and 0.199 ppm in Sari, 0.19 and >0.01 ppm in Noshahr, 0.163 and 0.088 ppm in Tonekabon, and 0.24 and 0.081 ppm in Ramsar. Since the water fluoride levels in these cities are lower than national standards for universal national and climatic conditions, it is recommended that public health authorities consider adding fluoride to the food chain of the citizens in these cities.

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Keywords: Fluoride in water; Noshahr, Ramsar, Sari, Tonekabon, Iran; Urban water distribution network.

### **SURVEY OF FLUORIDE LEVEL IN UNDERGROUND WATER SOURCE AND URBAN WATER DISTRIBUTION NETWORK IN QAEMSHAHR CITY IN NORTHERN IRAN FROM 2006 TO 2012**

Fluoride is considered by many health authorities to be one of the fundamental and required components of the human body. In underground water, because of its contact with fluoride-rich minerals and other sources, the fluoride concentration may reach several mg F ion/L. The recommended concentration of fluoride in drinking water is based on the regional temperature. In the current study, the status of fluoride in the underground water source and drinking water in Qaemshahr City in northern Iran is reported for the 21 March 2006 to 19 March 2012 and compared with national universal standards for climatic conditions. This is a descriptive and

sectional study. Water samples were selected randomly from active wells and the urban water distribution network from 1385 to 1390 hours. Samples were analysed for fluoride in the Qaemshahr water and wastewater department laboratory. Fluoride concentration was determined by DR2800 and SPADNS colorimetry. The maximum mean temperature in the different seasons was obtained from the meteorology department. Fluoride levels over several years were compared with each other and to national universal standards for climatic conditions. The minimum and maximum levels of fluoride in 419 samples of underground deep well water sources obtained during the years 2006 to 2012 were 0.01–0.67 ppm in spring, autumn, and winter, and 0.00–0.78 ppm in summer. Fluoride levels in 137 samples from the urban water distribution network were 0.06–0.68 ppm in spring and summer, 0.01–0.61 ppm in autumn, and 0.01–0.48 ppm in winter. Compared to WHO standards for various climatic conditions, the percentage of the recommended level of fluoride is 5–1% in spring, 18–7.6% in summer and 11–0% in autumn, and 14–0% in winter for the well water, and 0.7% in spring, 10–0% in summer, 8–0% in autumn, and 0% in winter for the urban distribution network water. According to these calculations, health authorities may wish to consider adding fluoride to the food chain for Qaemshahr citizens.

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Keywords: Fluoride in water; Iran; Qaemshahr City; Underground water; Urban water distribution network.

## FLUORIDE RESEARCH – FUTURE NEEDS AND PROSPECTS

Over the years since its founding in 1966 by Dr George L Waldbott (1898–1982), the International Society for Fluoride Research has been engaged in a broad range of significant and still developing activity in fluoride research. In 2002, the late Dr Richard G Foulkes (1923–2007) reviewed the impressive 35-year history of reports published in the Society's journal *FLUORIDE*, many of them based on presentations from more than 30 countries at ISFR conferences held during the last 45 years. Since the appearance of that review, there have been numerous further advances, especially in studies related to the action of fluoride on living systems and in the environment. Areas in which more research is needed include refinements in the accuracy, reliability, and ease of fluoride analysis and advances in methods and techniques for the study of effects of fluoride on biochemical pathways, endocrine gland function, enzyme activity, hard and soft tissue response in animals and humans, hormone activity and balance, interaction with vitamins and dietary supplements, neurological development, and oxidative stress. Synergistic effects of fluoride connected with genetic differences and toxic co-factors like aluminum, arsenic, lead, and radionuclides also require more study,

especially in long-term *in vivo* as well as *in vitro* research with appropriate controls and sufficient ranges of concentrations.

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Keywords: Advances in methodology; Fluoride research; Future needs; Long-term studies; Prospects ahead.

### **OSTEO-DENTAL FLUOROSIS IN DOMESTIC ANIMALS LIVING IN AREAS WITH HIGH FLUORIDE IN DRINKING WATER OF RAJASTHAN, INDIA**

Fluoride toxicosis in the form of osteo-dental fluorosis was observed in 443 immature and 2155 mature domestic animals living in areas of Rajasthan, India, with a high fluoride content (>3.0 ppm) in the drinking water. These animals included cattle (*Bos taurus*), buffaloes (*Bubalus bubalis*), horses (*Equus caballus*), donkeys (*E. asinus*), camels (*Camelus dromedarius*), sheep (*Ovis aries*), and goats (*Capra hircus*). No significant difference in the prevalence of osteo-dental fluorosis was found between mature and immature animals. However, mature animals showed relatively higher prevalence (28.4%) of skeletal fluorosis as compared with their younger counterparts (12.1%). Among these various kinds of animals, buffaloes showed the highest prevalence of dental (96.8%) and skeletal (66.9%) fluorosis, whereas goats showed the lowest (17.02% and 8.7%) prevalence. The maximum prevalence and severity of osteo-dental fluorosis was found in grass-eaters or grassers (cattle, buffaloes, horses, and donkeys) and the minimum in plant-eaters or browsers (camels, sheep, and goats). These differences were found to be statistically significant. Causes for difference in prevalence and severity of dental and skeletal fluorosis in different species of domestic animals are also discussed in this paper.

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Keywords: Domestic animals; Drinking water; India; Osteo-dental fluorosis; Prevalence of osteo-dental fluorosis; Rajasthan.

### **PROSPECTS FOR LOW-FLUORIDE WATER IN FLUOROTIC AREAS IN TANZANIA**

Tanzania is one of the richest countries in the world with respect to national parks. Most of the parks lie within the Great Rift Valley, where most fascinating wild life thrives abundantly. Throughout history, the lands within and around the parks have been sparsely populated by humans, probably because they have been considered marginal, without fresh water resources. Especially around the alkaline lakes, the surface waters as well as the groundwater sources contain unpalatable concentrations of the sodium carbonate-bicarbonate mineral Trona, locally called Magadi, along with highly toxic concentrations of fluoride (from 2 up to 90 mg F/L). Population growth and intensive modern tourism has made such areas very popular with which comes habitation of previously inhabitable land. The hunt for



fresh and safe water for human consumption is strongly ongoing and will probably be accelerating in the near and far future. Defluoridation of water by means of bone char is the process of choice. It is found that harvesting rainwater in combination with defluoridation of water for drinking and cooking may be the most appropriate solution to the fluoride problem in such areas.

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Keywords: Defluoridation of water; Magadi; National Park; Tanzania; Trona.

### **SIMULTANEOUS FLUORIDE AND LEAD EXPOSURE ACTING AS A PRO-INFLAMMATORY FACTOR IN DIFFERENTIATED HUMAN THP1 MONOCYtic CELLS**

Low-level, chronic exposure of humans to fluorine compounds (F) in the air, water, and food may be atherogenic via the activation of oxidative stress and increased production of reactive oxygen species (ROS). Exposure to lead (Pb) via the same trophic chain may potentiate the intensity of free-radical reactions and changes in lipid metabolism leading to induction and stimulation of the atherosclerosis progression. A long-term exposure can also lead to changes in the amount and catalytic properties of many enzymes, thereby enhancing the inflammatory and proliferation reactions in which a significant role is played by macrophages, which are cells that participate in the formation of atherosclerotic plaques. Early and key changes related to atherogenesis are implicated in oxidation modification of low-density lipoproteins (LDL). Macrophages are of prime importance in this process because of the accumulation of cholesterol originating from LDL and subsequent transformation into foam cells, being the source of locally secreted pro-inflammatory factors. These factors, acting simultaneously with excessive ROS synthesis, are the key ingredients of the development of the inflammatory- proliferative atherogenesis process. Glutathione peroxidase (GPx), by its reducing properties toward a wide range of hydroperoxides, influences the activities of the arachidonic acid metabolism enzymes cyclooxygenases (COX1 and COX2). GPx contributes to the detoxification of hydroperoxides and modulation of the synthesis of eicosanoids. The aim of this study was to investigate the influence of simultaneous fluoride and Pb exposure (in concentrations determined in human serum) on the activity of enzymes involved in eicosanoid metabolism: COX1, COX2 (by measuring the products of the enzymes prostaglandin PGE2 and thromboxane TXB2) and GPx1 activity in macrophages obtained from a monocytic line THP-1. THP-1 cells were differentiated into macrophages by administering phorbol myristate acetate (PMA). The THP-1 monocytes were treated with 100 nM PMA for 24 hr, and then the adherent macrophages were incubated for 48 hr with solutions containing NaF and lead acetate (PbAc) at final concentrations of 1, 3, 6, and 10  $\mu$ M NaF and 10, 100, 1000  $\mu$ M PbAc. These concentrations were selected on the basis of measurements of fluoride and lead levels in human serum from environmental

exposure. Simultaneous fluoride and Pb exposure increased the COX-1 and COX-2 activities in macrophages, resulting in the synthesis of the pro-inflammatory factors PGE2 and TXB2 and increased activity of GPx-1 in the macrophages. The observed changes of the enzymes activities occurring in these *in vitro* cell cultures, with implications for chronic *in vivo* effects in humans, were probably due to compensation from protection against excessive ROS generated during exposure to fluoride and Pb.

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### **FLUORIDE AND LEAD INFLUENCE ON APOPTOSIS PROCESSES IN THE LIVER CELL LINE HEPG2**

Chronic, long-term exposure to high levels of fluoride leads to fluorosis, which is manifested by skeletal impairment and damage to internal organs, including kidney, liver, parathyroid glands, and brain. Studies have shown that excess fluoride can cause DNA damage, trigger apoptosis, and change cell cycle. Additional effects of fluoride on the body can be superimposed on the effects of lead, which, when present in the environment in excessive amounts, is known to inhibit many enzymes and to cause apoptosis. Therefore, in the present study, we investigated the effects of sodium fluoride (NaF) and/or lead (Pb) on the development of apoptosis, on vitality and the index of proliferation in the liver cell line HepG2. We used hepatocytes from the liver cell line HepG2, which were incubated for 48hr with NaF, Pb, and mixtures of NaF+Pb for measurement of apoptosis, index of proliferation, and vitality of cells. Incubation of hepatocyte cells with NaF or Pb increased apoptosis, and this process was enhanced when F and Pb were used simultaneously. Vitality of the cells changed, depending on the compound used and its concentration. The index of proliferation slightly increased and then decreased in high fluoride environment, decreased significantly after lead addition in a dose-dependent manner, but when used together, fluoride abolished the decreasing effect of lead.

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Keywords: Apoptosis; Hepatocyte; Lead; Proliferation index; Vitality.

## **FLUORIDE CONTENT OF BOTTLED, DISTRIBUTION NETWORK, POINT OF USE DESALINATOR, AND DECENTRALIZED DESALINATION PLANT WATERS IN GONABAD, IRAN**

This study reports measurement of the fluoride (F) content of the 5 most available brands of bottled drinking water, distribution network water supply, 45 kitchen taps with under the sink point of use (POU) desalinator, and all 3 decentralized municipal (DM) desalination plants in Gonabad, Iran. Thin film composite (TCF) reverse osmosis (RO) and polyamide RO membranes were used in POU and DM desalination plants, respectively. The mean F concentration of samples in distribution network, kitchen taps POU, DM desalination plants, and bottled drinking water were 0.93, 0.4, 0.32, and 0.23 mg/L with a range of 0.83 to 1.11, 0.2 to 0.6, 0.12 to 0.43, and 0.09 to 0.63, respectively.

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Keywords: Bottled water; Decentralized municipal desalination; Distribution network water supply; Gonabad; Iran; Point of use desalinator; Water defluoridation.

## **ONE-YEAR EVALUATION OF CLASS-I COMPOSITE RESTORATIONS IN FLUOROSSED AND NON-FLUOROSSED PERMANENT TEETH**

Dental fluorosis is a developmental problem of tooth enamel, caused by chronic early intake of high levels of fluoride during tooth development. In many cases tooth enamel becomes opaque and porous and exhibits a mottled appearance ranging from white to yellow-brown and even black. Isparta is an endemic fluorosis area in Turkey. The aim of this study was to evaluate the clinical performance of a packable composite resin (Filtek P60, 3M-ESPE, USA) in fluorosed permanent molar teeth in adolescents according to modified Ryge criteria. A total of 145 Class I restorations were placed in permanent molar teeth of adolescent patients aged 12 to 17 years. Molar teeth were grouped according to Thylstrup-Fejerskov index (TFI) into normal (TFI 0), mild fluorosis (TFI 1–2), moderate fluorosis (TFI 3–4), and severe fluorosis (TFI 5–7). Restorations were assessed by two previously calibrated operators after the 3rd and 6th month and 1 year for color matching, marginal discoloration, secondary caries, marginal

adaptation, anatomic form, postoperative sensitivity, and retention. At the final appointment (after 1 year) 99.2%, 99.5%, 99.1%, 96.9% respectively of the restorations in normal, mild, moderate and severe fluorosis groups received Alfa ratings. A high percentage of optimal scores were recorded for secondary caries, anatomic form, and retention. For marginal adaptation, three restorations in the severe fluorosis group received a score of Beta (11.1%). The clinical performance of Filtek P60 packable composite resin was excellent after 1 year when used on fluorosed and non-fluorosed teeth. Nevertheless, we believe that long-term clinical and radiographical evaluations are necessary.

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Keywords: Clinical evaluation; Dental fluorosis; Packable composites; Permanent teeth.

### FLUORIDE EFFECTS ON OXYGEN CONSUMPTION AND OXIDATIVE STRESS IN RATS

Fluoride (F) increases bone mass but the presence of inflammation has been observed from F treatments in rats. The lack of effectiveness of F as an anti-osteoporotic drug could be due to inflammation. Inflammation could be the consequence of an increase in reactive oxygen species (ROS), causing modifications of the respiratory chain activity. The aim of this work was to evaluate the effect of F on the oxygen uptake rate ( $VO_2$ ) in rats treated with sodium fluoride (NaF). Eighteen Sprague-Dawley rats were divided into 3 groups (n = 6 per group): NaF20, NaF40, which received orally 20 or 40  $\mu\text{mol NaF}/100\text{g bw}/\text{day}$  for 30 days; and Controls that received only water.  $VO_2$  and fluoremia parameters were measured *in vivo* before and after 90 min of NaF doses. After 30 days, euthanasia was performed; plasma and mitochondrial glutathione peroxidase (GPx) and catalase (CAT) activities and TBARS concentrations were measured as indexes of oxidative stress. *In vitro*, the effect of F 100  $\mu\text{M}$  on the  $VO_2$  of liver slices and the isolated mitochondria was also evaluated. Finally, the effect of addition of 100  $\mu\text{M}$  of F on the activity of the respiratory chain complexes was assessed. Results are expressed as mean $\pm$ SEM and differences were considered significant when  $p < 0.05$  (\*). *In vivo*,  $VO_2$  ( $\mu\text{mol O}_2/\text{min}/100\text{g bw}$ ) decreased after 90 min of F dose in the NaF40 group (0 min:  $175.8 \pm 15.67$ , 90 min:  $156.0 \pm 16.62$ \*). Plasma and mitochondrial TBARS levels and GPx and CAT activities were higher in the NaF-treated groups. *In vitro*, F caused a decrease in the  $VO_2$  by liver slices ( $\text{nmol O}_2/\text{min}/\text{mg protein}$ ) (0  $\mu\text{M}$ :  $47 \pm 4.5$ ; 100  $\mu\text{M}$ :  $31 \pm 3.0$ \*) and by mitochondria ( $\text{nmol O}_2/\text{min}/\text{mg protein}$ ) in state 4 (0  $\mu\text{M}$ :  $55 \pm 5.0$ ; 100  $\mu\text{M}$ :  $29 \pm 3.5$ \*), and, in state 3 (0  $\mu\text{M}$ :  $146 \pm 9.7$ ; 100  $\mu\text{M}$ :  $87 \pm 9.3$ \*). The activity of complex I-III ( $\mu\text{mol}/\text{min}/\text{mg protein}$ ) was also inhibited by F (without F:  $1906 \pm 125$ ; with F:  $1720 \pm 71$ \*). Conclusions: F decreased  $VO_2$  *in vivo* after an oral dose, and the same effect was observed *in vitro* after the addition of F at concentrations similar to plasma levels. The effect of F on  $VO_2$  is observed only with high values of fluoremiias. F also decreased  $VO_2$  in isolated mitochondria. Thus, oxidative stress increased in the treatment with NaF. These results support the hypothesis that the

presence of inflammatory foci in bones of NaF-treated rats could be the consequence of an increase in ROS due to a modification of the activity of the respiratory chain.

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Keywords: Fluoride in rats; Inflammation; Oxidative stress; Oxygen uptake rate; Pro-inflammatory effects.

### **EFFECT OF SODIUM FLUORIDE ON BIOMECHANICAL AND HISTOMORPHOMETRIC BONE PARAMETERS: IDENTIFICATION OF VARIABLES THAT DETERMINE THE FRACTURE LOAD IN NaF-TREATED RATS**

Osteoporosis and tooth decay are very significant public health problems. For their treatment, sodium fluoride (NaF) and monofluorophosphate are the drugs containing fluoride (F) most commonly used. Disorganization of bone tissue and the presence of inflammatory foci observed when NaF is administered could be the cause of the lack of effectiveness of F in the treatment of osteoporosis. The aim of this work was to evaluate the effect of different F doses on biomechanical and morphometric properties of trabecular and cortical bone. Twenty-four Sprague-Dawley rats were divided into 4 groups (n = 6/group): NaF20, NaF40, NaF80, which received orally 20, 40, or 80  $\mu\text{mol NaF}/100\text{g bw/day}$  for 30 days. The Control group received water without NaF. After treatment, tibias and femurs were extracted. Histopathological and histomorphometric parameters were evaluated in the left tibia. In the right tibia, bone mineral density (BMD) and cortical parameters were determined. The femurs were used for biomechanical tests to evaluate trabecular and cortical bone properties. Results are expressed as mean $\pm$ SEM and differences were considered significant if  $p < 0.05$  (\*). Comparisons were performed using ANOVA test with Bonferroni's post test. Trabecular bone volume (%) was significantly decreased by the treatment with F (Control:  $23 \pm 2$ ; NaF20:  $21 \pm 2$ ; NaF40:  $18 \pm 1$ ; NaF80:  $14 \pm 2$ \*). Coincidentally, we observed a significant decrease in the trabecular fracture load (N) (Control:  $52 \pm 11$ ; NaF20:  $24 \pm 4$ ; NaF40:  $26 \pm 6$ ; NaF80:  $18 \pm 4$ \*) and Young's Modulus (GPa) (Control:  $0.16 \pm 0.04$ ; NaF20:  $0.08 \pm 0.02$ ; NaF40:  $0.07 \pm 0.02$ \*; NaF80:  $0.06 \pm 0.02$ \*) of NaF-treated groups. Cortical bone area ( $\text{mm}^2$ ) decreased significantly in NaF-treated groups (Control:  $4.1 \pm 0.19$ ; NaF20:  $4.0 \pm 0.14$ ; NaF40:  $3.8 \pm 0.09$ ; NaF80:  $3.4 \pm 0.07$ \*) and the three-point flexural test revealed a significant and a dose-dependent decrease in cortical bone strength (N) (Control:  $108 \pm 5$ ; NaF20:  $100 \pm 3$ ; NaF40:  $87 \pm 2$ \*; NaF80:  $84 \pm 4$ \*) and stiffness (Control:  $312 \pm 38$ ; NaF20:  $271 \pm 49$ ; NaF40:  $200 \pm 26$ ; NaF80:  $173 \pm 19$ \*). Apoptosis and inflammation were observed in the trabecular bone of NaF-treated groups. There were no significant differences in BMD between the groups. Finally, there was a significant correlation between the fracture load of cortical bone and bone volume, independently of Young's

Modulus. At the trabecular level, fracture load correlated with Young's Modulus but not with bone volume. These results indicate that cortical bone depends on the amount of tissue, but trabecular bone depends on its quality. It is concluded that chronically administered F as a single daily dose, produces significant decrease in biomechanical properties of bone by changing the quality and bone volume, although BMD is not affected.

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Keywords: Biomechanical properties; Bone quality; Fluoride and bone; Mice bones; Morphometric properties.

## **MID-TERM OVERVIEW OF JAPAN INTERNATIONAL COOPERATION AGENCY (JICA) FLUOROSIS MITIGATION PROJECT PHASE 2 (2011) IN SOUTHERN INDIA**

### **2. THE SURVEY AND PROPOSAL FOR SKELETAL FLUOROSIS**

In India, fluorosis is an endemic disease that is divided into three main categories: dental fluorosis, skeletal fluorosis, and nonskeletal fluorosis. It is estimated that more than 66 million people are suffering from this disease in 17 of 32 states in its country. According to the survey work by the director of health services in Dharmapuri in the Indian State of Tamil Nadu, approximately 6 million people appear to be classified with severe skeletal fluorosis. However, we doubt this survey report is based on plausible evidence. Members of our Department of Hygiene and Public Health at Osaka Medical College, Japan, were invited to participate in the project as expert consultants on fluorosis from the Japan International Cooperation Agency (JICA). Through our inspection we noticed that the accuracy of medical diagnosis was extremely low and many people were diagnosed with skeletal fluorosis only from their external deformity without clear diagnostic criteria. Moreover, the analysis of urinary fluoride, which is essential for correct diagnosis, was not performed at all, and no markers of bone metabolism were measured. As a result, a person who did not really have skeletal fluorosis was often diagnosed by mistake as a patient with skeletal fluorosis. At present, there is no fully effective treatment for skeletal fluorosis except by early detection or prevention. Therefore the supply of low fluoride or fluoride-free drinking water that comes from surface water is an urgent matter in this region. The output of medical approach in this JICA Fluorosis Mitigation Project Phase 2 is expected to improve the diagnostic skill and technique of health care workers (doctors, dentists, dieticians, and laboratory technicians) for confirming fluorosis. Based on the result of our checklist, however, strengthening medical facilities, maintenance of equipment, and training for special tests is not actually sufficient. It is necessary in the future that the infrastructure for fluorosis diagnosis should be further developed, and the equipment for measuring urine and blood fluoride and taking X-ray images should be better maintained, along with improved techniques and training of laboratory technicians.

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### **FLUORIDE PROMOTION OF VIABILITY AND DIFFERENTIATION OF OSTEOBLAST-LIKE SAOS-2 CELLS VIA BMP/SMAD SIGNALING PATHWAY**

Bone Morphogenetic Proteins (BMPs) are members of the TGF- $\beta$  protein family. BMP/Smad signaling pathways play important roles in the viability and differentiation of osteoblasts. Although the function of fluoride for osteoblast differentiation and viability has previously been characterized, the changes caused by fluoride in BMP/Smad signaling pathway in osteoblasts remain elusive. In this study, we investigated the role of BMP/Smad signaling pathway in fluoride-induced osteoblast-like Saos-2 cells differentiation. To determine whether the observed enhancement of Saos-2 cell differentiation by fluoride was mediated by increased proliferation or survival, we exposed Saos-2 cells to different concentrations of fluoride for 24, 48, and 72 hr at 0, 2, 4, 8, 16, and 32 mg F/L, measuring the cell number using a WST-based assay. To determine the effect of fluoride on osteoblast differentiation, Saos-2 cells were treated with 4 mg F/L for 72 hr, and real-time reverse transcription polymerase chain reaction (RT-PCR) was used to examine expression of genes involved in osteoblast differentiation, including bone protein gamma-carboxyglutamic acid (gla) among BGP and alkaline phosphatase (ALP). We found that fluoride enhanced the proliferation of Saos-2 cells in a dose-dependent manner with a higher expression of osteoblast marker genes. To further study whether Smads signaling acts directly on fluoride-induced differentiation of Saos-2 cells, we performed immunofluorescence against Smad1, Smad4, Smad5, and phosphorylated Smad1/5(p-Smad1/5) on Saos-2 cells during exposure to 4 mg F/L for 72 hr. Results showed that promotion effects of fluoride on Saos-2 cells differentiation were associated with the activation of the BMP/Smad signaling pathway. Expression of p-Smad1/5, as an indicator of BMP activity, was higher after stimulation of Saos-2 cells differentiation. Subsequently, we constructed the plasmid expression vectors encoding the short hairpin RNA (shRNA) targeting the Smad4 gene to block the BMP/Smad Signaling pathway, causing the expression of BGP and ALP detected by qRT-PCR to be significantly down transfected by Smad4-shRNA. Even after transfection, the expression of BGP and BALP was also significantly down stimulated by 4 mg F/L for 72 hr. The present results reveal that activities of the TGF- $\beta$  family were changed during the period of osteogenesis in a BMP/Smad pathway-specific manner, and that the activities of p-Smad1/5 required for Saos-2 cells differentiation were stimulated by fluoride.

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Keywords: Bone Morphogenetic Proteins; Fluoride and bone enzymes; Osteoblasts and fluoride; Saos-2 cells; Smad signalling pathway.

### **DETERMINATION OF FLUORIDE CONCENTRATION OF POWDER MILK IN IRAN 2010**

High concentration of Fluoride (F) in powder milk (formula-milk) can have adverse health effects on the body. The fluoride concentration of powder milk was analyzed in Iran in 2010. Twelve commercial brands of highly-consumed powder milk were selected to analyze fluoride content using a standard fluoride ion-selective electrode (ISC). Three samples with different production dates from each brand were selected. The mean  $\pm$ SD fluoride concentration in all the samples was  $1.73 \pm 0.3$  mg F/kg. The minimum and maximum F content in powder milk brands Humana2 and Humana3 were  $1.32 \pm 0.1$  and  $2.36 \pm 0.3$  mg F/g, respectively. The study revealed that there was no significant difference in fluoride concentration of the samples that belonged to various dates. Humana3 had high fluoride concentration (with an average of  $2.36 \pm 0.3$  mg F/g), which might be a risk factor for dental fluorosis, especially when mixed with water high in high F.

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Keywords: Baby; Powder milk; Teeth; Water for powder milk.

### **CHANGED CHOLINERGIC SYSTEM AND NEURONAL SIGNAL TRANSDUCTION IN RATS WITH DEFICIT OF LEARNING AND MEMORY INDUCED BY CHRONIC FLUOROSIS**

In order to reveal the mechanism of decreased learning ability and memory induced by chronic fluorosis, nicotinic acetylcholine receptors (nAChRs), cholinesterase (ChE) activity, and the pathway of extracellular signal regulated protein kinase (ERK1/2) were investigated using rats with chronic fluorosis from water pollution and coal-burning pollution. Spatial learning and memory of the rats were evaluated by Morris Water Maze test. The expressions of nAChRs, ERK1/2 and mitogen-induced extracellular kinase (MEK1/2) at protein and mRNA levels were detected by Western blotting and real-time PCR, respectively. ChE activity was determined by chemical colorimetry. The results showed that the learning and memory capacity in rats with chronic fluorosis was decreased. In the brain tissues from the rats with fluorosis as compared to controls, the protein expressions of nAChR subunits were lower, and the corresponding mRNAs of these receptor subunits changed; the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were reduced; increases of phospho-ERK1/2 and -MEK1/2 at the protein level but no change of ERK1/2 mRNA were found; and the activation rate of phospho-ERK1/2 was decreased. The modifications of nAChRs and ERK1/2 pathway might therefore be connected with the molecular mechanism



in the decreased ability of learning and memory of the rats influenced by chronic fluorosis.

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Keywords: Fluoride and learning deficit; Learning and memory; Nicotinic Acetylcholine Receptors; Rat learning; Signal transduction.

### **HYPERTENSION AND DRINKING WATER FLUORIDE – IS THERE A RELATIONSHIP?**

The correlation of drinking water fluoride and hypertension has been reported in an Iranian national-scale ecologic study (DOI: 10.1007/s12011-011-9054-5). Here we examined this hypothesis in the Larestan area of Iran in a regional-scale ecologic study. The data of hypertension prevalence by different gender in 2011—numbers of people which have blood pressure higher than 130/85—were obtained from health house records of 36 cities and villages. The annual mean 2010 data of drinking water fluoride in the corresponding cities or villages were also obtained from another study. Statistically significant negative correlations were found between the annual mean concentration of drinking water fluoride and hypertension prevalence of females ( $r = -0.411$ ,  $p = 0.013$ ) and overall ( $r = -0.372$ ,  $p = 0.025$ ). However, there was no clear association for males ( $r = -0.313$ ,  $p = 0.063$ ). In conclusion, our data indicated a decrease instead of an increase of hypertension prevalence with increased fluoride levels in drinking water. Therefore, more epidemiological and experimental studies are recommended on this matter with better control of confounding factors.

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Keywords: Blood pressure; Ecologic study; Fluoride and hypertension; Hypertension; Larestan area, Iran.

### **THE INFLUENCE OF LIPOIC ACID AND VITAMINS A AND E ON SELECTED BIOCHEMICAL PARAMETERS IN THE KIDNEYS OF RATS INTOXICATED WITH SODIUM FLUORIDE**

Previous studies have shown that exposure of rats to sodium fluoride has detrimental effects on tissues and cells. Significant impact of fluoride on pathomorphological changes in organs is largely associated with the interference in oxidative processes. The addition of some substances to the diet can reduce the consequences of the NaF-induced oxidative stress. The main purpose of this research was to assess the influence of lipoic acid and antioxidant vitamins A and E on selected enzymes associated with protein metabolism and their impact on the

morphological picture of kidneys of rats intoxicated with sodium fluoride. The study was conducted on 24 adult female Wistar rats. The animals were divided into 4 groups. The control group received distilled water. Rats in the study groups (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>) were orally administered NaF at a dose of 10.0 mg/kg bw/24 hr. Rats from the 2<sup>nd</sup> group were additionally administered  $\alpha$ -lipoic acid at a daily dose of 70 mg/kg bw. The 3<sup>rd</sup> group was administered vitamin E at a daily dose of 3 mg per rat, vitamin A at a daily dose of 250 IU per rat, and  $\alpha$ -lipoic acid at the dose above. The experiment lasted for 7 weeks. Afterward, the rats were intraperitoneally anesthetized with thiopental at a dose of 30 mg per rat. At autopsy, the kidneys were removed for histopathological examination and some fragments of organs were collected for biochemical analyses. Ten percent of these parts served as homogenates, in which the activity of protein metabolism enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and  $\gamma$ -glutamyltranspeptidase (GGTP), were determined. Total protein concentration and total antioxidative status (TAS) were also estimated. Slides made by the normal paraffin method, stained with hematoxylin and eosin (H-E), were prepared to assess pathomorphological changes in the organs. Sodium fluoride exposure of rats had detrimental effects on the biochemical parameters examined. A statistically significant increase in the activity of GGTP, AST, ALT, and a decrease in TAS was noted in kidneys in the group exposed to NaF. The administration of  $\alpha$ -lipoic acid in combination with or without vitamins A and E, during simultaneous administration of NaF, caused positive changes in the activity of AST and ALT, and it also re-established the concentration of TAS in kidneys at the level in the control group. The regressive changes and circulation disorders accompanied the biochemical changes observed after exposure to fluoride. As seen here, co-administration of antioxidants had a positive influence on morphological changes in kidneys that resulted from exposure to fluoride.

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## REMOVAL OF FLUORIDE FROM AQUEOUS SOLUTION BY BIOSORPTION ONTO *SARGASSUM* BIOMASS

Fluoride pollution of water has become a problem due to leaching of fluoride from certain minerals by rainwater and from human activities through different industrial applications. Among the several methods for the removal of fluoride from water, adsorption is an effective technique. In the present study, dried algal biomass of *Sargassum* (a type of marine algae) was used to biosorb fluoride from synthetic solutions. The effect of various parameters such as initial fluoride concentration, dose of biosorbent, contact time, and pH on the efficiency of the adsorption process was investigated. It was found that the sargassum biosorbent

adsorbs fluoride effectively. The adsorption efficiency was dependent on initial fluoride concentration and amount of biosorbent and followed the Langmuir isotherm adsorption model. Results also showed that the aqueous phase pH significantly affected the efficiency of the adsorption process with the optimum pH for fluoride sorption being acidic. These findings showed *Sargassum* algal biomass is an inexpensive adsorbent with a considerable potential for biosorption removal of fluoride from aqueous solutions.

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Keywords: Algal biomass; Biosorption; Fluoride removal; *Sargassum* algae.

## EVALUATION OF FLUORIDE CONTENT IN SANANDAJ BOTTLED WATER, IRAN

This study aimed to measure the fluoride content in bottled waters sold in the city of Sanandaj, Iran, and to compare the results with fluoride levels on their labels and with national standards. Ten brands of bottled water were sampled randomly over two seasons from Sanandaj markets. Ion chromatography was used to measure the fluoride ion concentration. ANOVA, POST HOC, and Pearson tests were used for statistical analysis with SPSS software, Version 11.5. Results show that the fluoride ion concentration in different brands had significant differences ( $p < 0.05$ ) with a mean level of  $0.157 \pm 0.0958$  mg/L. Among eight brands for which the fluoride ion concentrations were given on their labels, four differed significantly from the measured values. Overall, the measured levels did not match well with the values on the labels. The reliability coefficient of the measured fluoride ion concentration in all brands was less than the lower range of Iranian standards for fluoride (0.7–1.2 mg/L).

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## EXPERIMENTAL DESIGN AND RESPONSE-SURFACE MODELING FOR OPTIMIZATION OF FLUORIDE REMOVAL FROM WATER BY *MORINGA OLEIFERA* SEED EXTRACT

In addition to other technologies for removal of excess levels of fluoride from water, coagulation has often yielded efficient results. Among different chemical coagulants, iron and aluminum salts have been widely applied for pollutant removal. The limitations of such coagulants, including problem of their handling,

high volume of generated sludge, and requirements of initial and final pH adjustment, have encouraged researches to look for new coagulants. *Moringa oleifera* has been considered a natural and efficient coagulant with some benefits. In this study, fluoride removal using *Moringa oleifera* seed extract was investigated. Optimum conditions were determined and modeled using response-surface methodology. The Box-Behnken method with three variables (coagulant, pH, and initial fluoride concentration) and three levels of seed extract were applied. At constant initial fluoride concentration, with increase in *Moringa oleifera* dosage, fluoride removal increased. It was also shown that pH had no significant effect on fluoride removal. Under optimal conditions, high levels of fluoride can be significantly reduced.

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Keywords: Coagulation; Defluoridation of water; *Moringa Oleifera*; Optimization of defluoridation; Response surface.

## TOPICAL FLUORIDE IN PEDIATRIC DENTISTRY

Topical application of highly concentrated forms of fluoride has been provided in clinical settings for 50 years. The most commonly used agents have included 8% to 10% solutions of stannous fluoride (now no longer used) as well as 2% sodium fluoride and 1.23% acidulated phosphate fluoride (APF). Numerous studies conducted prior to 1980 reported caries reductions averaging approximately 30% following use of these agents. More recently, fluoride varnish has become a popular topical agent for preschool and school-age children and children with special health care needs, and has been recommended for decalcified enamel. An electronic literature search of published scientific papers in Cochrane Library, MEDLINE, and PubMed based on topical fluoride was carried out for the period 1965 to 2011, and practical and clinical reports were evaluated. Recommendations classified by age groups and caries risk show that periodic fluoride treatments should be considered for both children and adults who are at moderate or high risk of developing caries. The dentist, knowing the patient's health history and susceptibility to caries, is in the best position to make treatment decisions in the interest of each patient. These clinical recommendations must be balanced with the practitioner's professional expertise and the individual patient's preferences. Children whose teeth have structural defects or exhibit decalcified areas or other indications that place them at moderate or high risk for developing caries, or as infants who have experienced severe caries, may be advised to receive

additional topical applications in the form of a professionally administered preparation (e.g., APF gel or fluoride varnish) or a concentrated preparation given to them by their parents.

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Key words: Caries prevention; High caries risk; Pediatric dentistry; Topical fluoride.

### **EFFECT OF SUPPLEMENTATION OF SLIMMING PREPARATIONS CONTAINING CONJUGATED DIENES OF LINOLEIC ACID (CLA) ON THE FLUORIDE, CALCIUM, AND MAGNESIUM CONTENT OF HARD TISSUES AND SERUM IN MICE**

In many countries, the need for treatment of obesity has become a significant problem. Increasing numbers of patients with obesity, especially with visceral type of obesity, have developed a growing number of obesity complications. In order to meet the market demand for slimming or weight-loss diet supplements, diet supplements supporting weight loss have focused on the increasing the content of certain types on unsaturated fatty acids in fat tissue. Since the role of conjugated linoleic acid (CLA) in weight loss has been documented, the main ingredients of those products are conjugated CLAs. Diet supplements containing CLA are usually a mixture of two linoleic acid isomers, *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA, present in an equal 1:1 proportion. Each of the isomers can exert an opposite biological action. However, only the *trans*-10, *cis*-12 CLA is the active agent in the adipocyte delipidation process *in vivo* leading to reduction of fat tissue. The second isomer, *cis*-9, *trans*-11, does not exert a delipidation effect, either *in vitro* or *in vivo*. The actual influence of CLAs on body mass reduction has not been clearly explained. Moreover, medical studies have not provided data on the influence of CLAs on micro- and macroelement metabolism in various tissues. We decided, therefore, to examine the influence of CLAs on fluoride accumulation in hard tissues of mice. In this research we used the hard tissue bones and teeth and the serum of 6-week-old mice fed a MURIGRAN fodder that included 6.5% soy olive (control group) or 5.5% soy olive with 1% CLA supplementation (experimental groups: *cis*-9, *trans*-11 CLA; *trans*-10, *cis*-12 CLA; CLA mix 1:1; LA) during four weeks. Fluoride concentration was determined with an ion selective electrode and calcium and magnesium concentrations by atomic absorption spectrometry. The results of the study were analyzed with the Mann-Whitney U test with significance set at  $p = 0.05$ . Supplementation with the various isomers of linoleic acid (*cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, CLA mix 1:1, LA) caused changes in bone and teeth mineral composition in the mice. The greatest changes were observed with *cis*-9, *trans*-11 CLA, which does not exert a delipidation effect either *in vitro* or *in vivo*, but is added to slimming diet supplements in 1:1 proportion.

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Keywords: Bone health; Calcium; Conjugated linoleic acid dienes; Delipidation; Dietary magnesium; Obesity and fluoride; Slimming diet supplements.

### **FLUORIDE INFLUENCE ON ENERGY SYNTHESIS IN SKELETAL MUSCLE CELL LINE CCL136**

It is well known that fluoride, even at a low concentration but with a long-time exposure, accumulates in the body and leads to numerous metabolic disorders. Many authors have reported studies on the influence of fluoride on the activity of various enzymes taking part in different metabolic pathways. However, there are only few reports about the influence of fluoride on glycogen metabolism in the muscle, which is a very important source of glucose and energy for muscle work. We therefore decided to examine the energy synthesis in skeletal muscle after addition of fluoride to skeletal muscle cells from cell line CCL136, which were incubated for 48hr with 1, 3, 6, and 10 OM NaF and used for measurement of ATP synthesis, glycogen accumulation in the cells, and expression of enzymes taking part in glycogen metabolism. Increased accumulation of glycogen occurred in the skeletal muscle cells, which suggested glycogen phosphorylase activity might have decreased. This was confirmed by measurement of the expression of glycogen phosphorylase taking part in glucose release from glycogen. This inhibition of glucose release in turn causes decrease in the synthesis of cellular ATP.

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### **FLUORIDE IN THE BRAIN AND PINEAL GLAND OF TWO DUCK SPECIES, AYTHYA FULIGULA AND MELANITTA FUSCA, WINTERING IN NORTHWESTERN POLAND**

Fluoride (F<sup>-</sup>) deposits formed, e.g., in bones, have hitherto mainly been analyzed in highly mineralized tissues obtained from mammals and only rarely from birds. Under certain conditions, F<sup>-</sup> may also accumulate significantly in the brain and

pineal gland and interfere with the secretion of melatonin by that gland. The mammalian and avian pineal gland is called the fifth mineralizing tissue, since it is one of the tissues in the body in which calcification occurs physiologically. In the pineal gland of the elderly, remarkably elevated  $F^-$  levels occur, reaching as high as 875 mg/kg gland weight and in the pineal calcifications  $>20,000$  mg/kg. However, fluorides have not yet been measured in brain and pineal gland of wild ducks. The aim of our study was to assay and compare  $F^-$  concentrations in the brain and the pineal gland in adult ducks of two species, one wintering in the Odra estuary (tufted duck, *Aythya fuligula*,  $n = 44$ ) and the other in the Pomeranian Bay (velvet scoter, *Melanitta fusca*,  $n = 9$ ) of northwestern Poland. The material was collected during the years 2008–2010.  $F^-$  was determined by a potentiometric method (using the Orion electrode) and expressed as concentration in dry weight, with the assumption that the duck brain contains 79%  $H_2O$ . In the tufted duck, medians of  $F^-$  concentration in the brain and pineal gland were 203 mg/kg (range 77–593 mg/kg) and 2,360 mg/kg (range: 674–38,645 mg/kg), respectively. In the brain and pineal gland of the velvet scoter, medians of 174 mg  $F^-$ /kg (range: 93–551 mg/kg) and 462 mg  $F^-$ /kg (range: 176–2,269 mg/kg) were found, respectively. In contrast to  $F^-$  levels in the pineal gland,  $F^-$  levels in the brain appeared to show significant differences between the two duck species.

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## PREVALENCE, PERCEPTION, AND TREATMENT ALTERNATIVES OF DENTAL FLUOROSIS IN A TURKISH RURAL COMMUNITY

The purpose of this study was to determine the fluoride concentration of drinking water in Deregümü village, Isparta, Turkey, and to correlate the frequency, severity, and discoloration level of dental fluorosis and the perception of it by individuals. Water samples were taken from the drinking water tank of the village and from the groundwater source. Intraoral examination was made of 283 individuals (140 females, 143 males) between 12 and 80 years of age. The female and male individuals were divided into four age groups. The Thylstrup-Fejerskov Index (TFI) dental fluorosis scores and discoloration levels were recorded for each tooth, and individual perception was determined by a questionnaire. Twenty appropriate volunteers were selected for office and home bleaching treatments. Statistical analysis was performed using the Kruskal-Wallis, Mann-Whitney, Bonferroni-Dunn, and Spearman rank correlation tests. The prevalence of dental fluorosis in this population was 94.1%, with TFI scores between 1 and 8. The mean level of fluoride in the drinking water was 1.83 mg/L. The mean TFI score

was 3.58. Discoloration was severe (score of 3) in 25.08% of the individuals, moderate (score of 2) in 20.14%, and mild (score of 1) in 20.5%, whereas 34.28% had no discoloration (score of 0). TFI and discoloration scores were positively correlated with age in both genders, whereas they were positively correlated with gender in some age groups ( $p < 0.05$ ). Young individuals and females had higher awareness than males and older individuals who requested bleaching therapy. Satisfying results were achieved in bleaching discolorations. Because of the very high prevalence and relatively severe degree of dental fluorosis in the population of Deregümü, Isparta, Turkey, residents should be informed about preventive options and treatment alternatives.

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### **NUTRITIONAL, CLINICAL, AND BIOCHEMICAL STATUS IN AN 8-PPM FLUORIDE WATER VILLAGE IN THE NALGONDA DISTRICT OF ANDHRA PRADESH, INDIA**

Fluorosis is a major public health problem in India and is aggravated by malnutrition. The present field study, a community-based cross-sectional study, was undertaken to investigate the role of nutrition in fluorosis in a fluoride-affected and a normal village in Nalgonda, a rural district of Andhra Pradesh, India. The study population included 182 randomly selected individuals between age 15 and 55 years (age and sex matched) from the fluorotic village of Venkapalli (water F = 8.00 ppm) and 62 from the nonfluorotic village of Rampalli (water F  $\leq$  1.00 ppm). Data were collected on all individuals for clinical examination, X-ray, DXA, diet survey, blood and urinary fluoride. Dental and skeletal fluorosis were significantly higher in the affected village than in the control village, and serum F and total and bone-specific alkaline phosphatase (ALP) was significantly higher in the affected village than in the control village. X-rays showed narrowed joint space, osteoporosis, and formation of osteophytes. DXA indicated that, in the spine area, BMC and BMD were significantly higher in the high-fluoride village, as was urinary fluoride, than in the low-fluoride control village. Serum 25(OH) vitamin D, PTH, TRAP were significantly lower in all age groups of the affected village than the control, whereas 1,25(OH)<sub>2</sub> vitamin D was significantly higher compared to the control. The diet survey showed there was significantly higher intake of milk and milk products and nutrients like folic acid, calcium, and phosphorus, in the fluorotic village as compared to the control village. DXA showed a higher trend (not statistically significant) for total fat and fat percent in the control and non-affected group in the fluorotic village than in the affected group in the fluorotic village. Mean body mass was higher in non-affected subjects than in the affected individuals and in the control group. Hemoglobin was significantly lower in the fluorotic village than in the control village. Serum T3 level was significantly higher in the affected village than in the control village, whereas T4 and TSH were comparable in both the affected and non-affected



villages. In conclusion, the effect of high fluoride water was less than observed elsewhere at this concentration, apparently owing to better nutritional status in the fluorotic village.

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Keywords: DXA; Effects of diet; Fluorosis in India; Fluoride toxicity; Nutrition and fluorosis; Vitamin D; X-Ray.

### **ADSORPTION OF FLUORIDE FROM AQUEOUS SOLUTION BY MODIFIED FLY ASH**

Novel adsorbents, aluminium- and iron-modified fly ash (AMA and IMA), were prepared by wet impregnation of fly ash with aluminum sulfate and ferric sulfate solutions for adsorption of fluoride from aqueous solution. Physicochemical properties of the adsorbents were characterized by X-ray diffractometry, scanning electron microscopy, and X-ray fluorescence spectrometry. Fluoride adsorption experiments were conducted to test the effects of pH, adsorbent dosage, and contact time at room temperature. Since the chemical quality of groundwater generally varies from place to place, the maximum adsorption capacities for AMA and IMA were 3.04 and 2.73 mg/g, respectively as calculated by the Langmuir isotherm model. Of the two adsorbents, AMA was more effective than IMA for removal of fluoride from aqueous solutions. The adsorption efficiency of fluoride onto both AMA and IMA was found to increase with contact time and amount of adsorbent, and with decreasing pH and concentrations of the sulfates in the adsorbate. Coexisting ions had a diverse impact on the adsorption rate of fluoride by AMA and IMA in the order: comprehensive > bicarbonate > chloride > sulfate. This study indicated that removal of fluoride from water by aluminium- and iron-modified fly ash is a low cost and promising technique.

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### **FLUORIDE IN ALCOHOLIC DRINKS**

Alcoholic drinks are important in the diet of many people and are the main source of exogenous ethyl alcohol. Due to the prevalence and extensiveness of alcohol consumption, alcoholic drinks should also be treated as a potential source of other xenobiotics that should be taken into account when balancing and assessing diets. In view of the continuing controversy regarding the use of fluorine compounds for the prevention of dental caries and the related risk of adverse effects (fluorosis), the determination the fluoride content in elements of the diet, including alcoholic drinks, is of utmost importance. The relatively small number

of papers reporting fluoride levels in alcoholic drinks (apart from wine) has caused us to assess alcoholic drinks as a potential source of dietary fluoride. The aim of the study was to determine the role of alcoholic drinks as a potential source of fluoride in the diet. Fluoride levels in alcoholic drinks with alcohol contents of less than 10% and between 10 and 20% were significantly higher, statistically, than in drinks with alcohol contents of 20–40% and above 40%. In terms of types of alcoholic drinks, fluoride levels in both beers and wines were significantly higher than in vodkas. Due to the fact that low-percentage alcoholic drinks (beers, wines) are consumed in significantly larger quantities than high-percentage alcohols (rums, vodkas), they should be considered a source of significant amounts of dietary fluoride.

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Keywords: Alcoholic drinks; Dietary alcohol; Fluoride in alcoholic drinks.

### **RISK OF FLUORIDE COMPOUNDS IN THE WORKFORCE: THREE OCCUPATIONAL EXPOSURE CASES**

Currently in Japan, there are about 20 fatal occupational accidents per year due to exposure to toxic substances. Since they are only a tiny percentage (< 2%) of all the fatal occupational accidents of >1,000 per year, the risks posed by toxic substances in occupational setting have not been fully recognized. Fluoride compounds are used in a wide range of applications throughout downstream industries. Here we describe occupational fluoride compound intoxication cases caused by HF inhalation exposure, contact with HF solutions, and inhalation of 1,1,2,3,3-pentafluoro-3-chloropropene (Perfluoroallyl chloride, PFAC). A 52-year-old worker manufacturing electric condenser cylinders showed severe dyspnea after metal-surface cleaning with hydrofluoric acid. On arrival at the emergency medical center, X-ray of both his lungs and computed tomography (CT) image showed a fine diffuse veiling over pulmonary fields due to HF chemical pneumonia. Laboratory tests revealed severe hypokalemia (8.1 mg K/dL), high serum F (74.6 µg/dL) and high urine F (80 mg/L). He was immediately given 5% calcium gluconate solution by intermittent positive-pressure breathing (IPPB), utilizing a nebulizer. He was discharged on the 22<sup>nd</sup> hospital day in a much-improved condition. A 65-year-old worker suffered a third-degree skin burn to 5% of his total body surface area with HF solution that splashed in his face during maintenance of HF liquefying tank pipes. He died shortly thereafter without having received adequate first aid. His serum fluoride concentration was markedly increased at 6.38 µg/dL with hypocalcemia and hyperkalemia. Fluoride ions from HF can be absorbed into the body, rapidly deplete calcium and result in hypocalcemia. With the onset of clinical hypocalcemia, heart function is impaired and results in cardiac dysrhythmia. Calcium gluconate is very effective and the only HF detoxification drug. A 39-year-old man was accidentally exposed to

PFAC gas while performing chemosynthesis of polymer electrolyte membrane fuel cell (PEMFC). He felt symptoms such as a cold 1-2 hr after he conducted the experiment. When he was admitted to the hospital, his chest X-ray and computed CT image revealed evidence of pleuritis. Although pulse steroid and antibiotics therapy was started, he expired on the 18<sup>th</sup> post-exposure day with severe pneumothorax. There is no direct evidence that he was exposed to PFAC gas, and the specific mechanism of PFAC toxicity still remains poorly understood except that fluorocarbon derivatives cause flu-like symptoms (polymer fume fever). However, circumstantial evidence of the onset of symptoms, diagnosis of chemical pneumonia, and his entire clinical course strongly suggest a PFAC gas exposure accident. The present cases indicate that personal protective equipment is essential, and an adequate method of emergency first-aid treatment for accidental poisoning is necessary.

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## MID-TERM OVERVIEW OF JAPAN INTERNATIONAL COOPERATION AGENCY (JICA) FLUOROSIS MITIGATION PROJECT PHASE 2 (2011) IN SOUTHERN INDIA 3. DENTAL APPROACH

In the southern India state of Tamil Nadu, groundwater is seriously polluted with fluoride. Due to consumption of fluoride-contaminated water during everyday life, many of the inhabitants in this district are suffering from the world's oldest known disease, fluorosis, but its actual prevalence there has remained unknown. Since 2008 the Japan International Cooperation Agency (JICA) has provided assistance to reduce fluoride contamination in drinking water under the Hogenakkal Water Supply and Fluorosis Mitigation Project to be completed by 2014. Osaka medical college dispatched Japanese experts on this project for an ad hoc medical, dental, chemical, and nutritional survey. In 2011 our dental experts carried out the evaluation for the implementation of basic education policy to prevent dental fluorosis through hospital inspections and face-to-face screening examination for dental fluorosis prevalence at various Tamil Nadu villages as the project mid-term progress review. Our team confirmed that the training manual booklet for doctors, the school children dental program, and dental fluorosis identification card were provided for the project participants. These manuals and modules were developed by joint work of JICA, a non-profit organization (NPO) of the Center for Symbiosis of Technology Environment and Management (STEM) and the Tamil Nadu Water Supply and Drainage Board (TWAD). Although the contents of these manuals and modules were essentially adequate and appropriate, their effective

promotion and distribution for utilization will be a key element of future project success plan. Our team visited several primary health centers (PHC) and high schools to assess the prevalence of dental fluorosis among school children and village inhabitants exposed to fluoride contaminated public water supplies. Although the current village population status or school student population is unknown, our team conducted face-to-face screening examination for dental fluorosis and there was an impression that the condition had a prevalence of about 80%. Brown teeth (tooth stains) also can be caused by poor oral hygiene, foods, and drinks. Many other kinds tooth stains are similar in appearance to dental fluorosis. Conditions such as these must be ruled out before a reliable diagnosis of dental fluorosis can be made. Our screening examination also gained the impression of fairly low dental caries prevalence among the school children. The dental caries preventive effect of fluoride may contribute to this condition and a necessary action plan should be undertaken after the establishment of new waterworks and services with fluoride-free or low-fluoride water. A serious shortage of trained personnel and equipment at government general hospitals made it difficult to conduct the baseline survey of dental fluorosis or accurate diagnosis. A strategic increase in medical personnel and medical facilities will be essential for strengthening the project activities. Since the historical fact from progress in the developed world revealed that water supply network systems helped solve the problem of dental fluorosis, our mid-term review team found that water supply network distribution, closing the demand supply gap in health manpower, and dental fluorosis prevention campaign are urgent requirements to achieve the project's goals by the year 2014.

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### **THE EFFECT OF THE PERIPARTURIENT PERIOD ON PLASMA FLUORIDE CONCENTRATIONS IN GOATS (*CAPRA HIRCUS*)**

Pregnancy and the periparturient period are known to modify metabolism in the maternal organism. Many organs as well as the nervous and endocrine systems are highly mobilised in order to provide optimal conditions for embryonic development. During pregnancy, maternal tissues are involved in providing energy for reproductive processes potentially influencing the concentrations of metabolites in the blood, which are also affected by several other factors such as breed, age, energy balance, fetal growth or season. On the other hand, the etiology of osteopenia and osteoporosis in a goat's infant kids has not been fully explained. Chronic deficiency of minerals can decrease the formation of the osseous matrix and tissue mineralization. Ketosis, which can occur during pregnancy, is an

additional factor that also intensifies the resorption of the osseous tissue through the activation of osteoclasts. This may lead to changes in the concentration of not only biogenic elements in this tissue such as calcium and magnesium, but also fluorine, which is considered to be one of the strongest stimulators of osseous tissue formation. Taking these facts into consideration, we aimed to examine the effect of the periparturient period on plasma fluoride concentrations in goats (*Capra hircus*); 75% genotype of Polish Noble White goats, aged 2–3 years and weighing 45–55 kg. Blood samples were taken from 12 goats during the periparturient period (4 and 1 weeks before and 2, 10, and 30 days after delivery), and from 10 nonpregnant goats. The fluoride concentration in plasma was determined by the potentiometric method. Pregnant goats were observed to have a statistically significant lower concentration of fluorine in plasma compared with the nonpregnant goats. Moreover, the concentration of fluorides in the plasma decreased after delivery.

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Keywords: *Capra hircus*; Goats; Fluoride and pregnancy; Periparturient period; Plasma fluoride.

## **FLUORIDE, CALCIUM, AND MAGNESIUM IN THE HAIR OF YOUNG MEN OF THE BANTU LANGUAGE GROUP IN TANZANIA VERSUS ENVIRONMENTAL AND SOCIAL CONDITIONING**

Chronic long-term exposure to fluoride is known to cause fluorosis. People living in areas contaminated by fluoride accumulate fluoride predominantly in hard tissues. The analysis of the content of chemical elements in hair is an important tool in toxicological and ecological investigations, and it has become one of the most studied biomarkers. However, research to determine their content in the hair of black populations is relatively limited. We are not aware of any hair analyses of black populations living in an indigenous environment like the Mafinga region, Iringa District, in Tanzania. The aim of this study was to analyse the content of F, Ca, and Mg in this easily available biological source. The hair samples were collected between October 2005 and March 2006 during anthropological research and came from young male students ( $n = 52$ ) in a secondary school. Due to the broad age range of the subjects (12.94–18.75), they were classified into two age groups: a younger group <16 years old ( $n = 26$ ) and an older group 16 years old ( $n = 26$ ). Hair samples were obtained from students who did not have dyed or treated hair. The concentrations of Ca and Mg were analyzed by atomic absorption spectrometry. Fluoride was determined using an Orion fluoride-ion-selective electrode (Thermo Scientific, USA). A fairly large Ca concentration range was found for the total number of subjects (854.1 to 1290.6  $\mu\text{g/g}$  dry matter (dm) with a mean value for the younger group of  $1080.5 \pm 145.30$   $\mu\text{g/g}$  dm and  $1038.11 \pm 118.75$   $\mu\text{g/g}$  dm for the older group. The mean Mg content

in the hair of the younger group was  $6.758 \pm 1.895 \mu\text{g/g dm}$  and  $5.848 \pm 1.762 \mu\text{g/g dm}$  in the older group. The mean F concentration for the older group, however, was significantly higher than in the younger group:  $103.563 \pm 79.501 \mu\text{g/g dm}$  vs.  $53.009 \pm 29.339 \mu\text{g/g dm}$  ( $p = 0.0055$ ). The hair fluoride concentration was positively correlated with age ( $R_s = 0.38$ ,  $p = 0.008$ ). Body mass index (BMI) was calculated for the subjects showing that out of 52 young males participating in the study, 9.6% were characterised by mediocre nutrition ( $\text{BMI} \leq 18.49$ ). No statistically significant correlations were found between BMI and the analysed chemical elements. The analysis of the chemical elements in the hair of the examined young males showed deficiencies in Mg and low concentration of fluoride.

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Keywords: Bantu language group; Calcium in hair; Fluoride in hair; Hair analysis; Magnesium in hair; Tanzania.

### **WATER FLUORIDATION, DENTAL FLUOROSIS, BONE FLUOROSIS, AND SKELETAL FLUOROSIS AMONG PERSONS IN THE HOJAI SUB-DIVISION, NAGAON DISTRICT, ASSAM, INDIA: A QUANTITATIVE OVERVIEW**

The aim of this project was to study the prevalence and severity of dental fluorosis, bone fluorosis, and skeletal fluorosis among persons exposed to high fluoride drinking water in Assam, India, and to assess the risk factors involved. Subjects aged 5 to 50 years who had been lifelong consumers of high-fluoride groundwater ( $=10\text{--}22 \text{ mg F/L}$ ) were selected for the study. The ground water samples were collected in three seasons over one year in 2010–2011 from 50 different sources in 30 locations in the predominantly rural and tribal area of the Hojai Sub-Division of Nagaon district, Assam, India. The nature of the fluoride sources with their approximate depth were tube well, ring well, pond, and other source water. Fluoride concentrations in the water samples were determined using the SPADNS method. Among the consumers of high-fluoride water, nearly 100% exhibited dental fluorosis, and 80% in the group consuming high-fluoride water were suffering from bone fluorosis. Sources, distribution, and geochemistry of fluoride are also studied. In the study area, which was characterised by multiple fluoride sources, uncontrolled high-fluoride groundwater was the most important factor in the development of dental fluorosis and skeletal fluorosis. In order to prevent fluorosis, groundwater wells should be analysed routinely for fluoride. Fluoride concentrations in the groundwater have also been correlated with those of other ions. With the high level of fluoride in the groundwater in various parts of the Hojai Sub-Division, fluorosis among the children and adults is evident even in photographs. The only effective way to prevent this disease is to stop the

consumption of fluoride-contaminated water. Because of continuous exposure to these high fluoride levels in the water, children examined in 2010–2011 appeared to be at higher risk for both dental fluorosis and bone fluorosis in the various tribal communities.

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Keywords: Assam, India; Bone fluorosis; Dental fluorosis; Fluoride contaminated water; Hojai subdivision, Nagoan district.

### **TEMPORAL AND SPATIAL MEASUREMENT OF FLUORIDE IN DRINKING WATER IN ARGENTINA**

Fluoride is found in nature in water, rocks and salts. It is also used in industry and is involved in human health with dose-dependent toxic effects. The upper limit in drinking water is 1.5 ppm as recommended by the World Health Organization. The aim of the present work was to describe the levels of fluoride in groundwater of Santa Fe, Argentina, and to evaluate changes over time and distance. Water from wells was collected from different areas, and the fluoride concentration was found to be below the upper limit in 72% of the towns. The comparison of this study with a previous one made in 1945 revealed an increased fluoride concentration in some areas. All values are shown as mean $\pm$ SEM and expressed as ppm. The linear regression of fluoride concentration in 1945 as a function of fluoride concentration in 2011 was significantly lower than 1 (slope =  $0.67\pm 0.072$ ,  $p < 0.05$ ), indicating an increase in the amount of fluoride in well water through the intervening decades. An important variability among the wells of the same town was observed, independently of the distance between them. For example, three wells in the city of Venado Tuerto show the following differing fluoride concentrations with ANOVA  $p < 0.05$ : well 1:  $1.92\pm 0.06$ , well 2:  $2.80\pm 0.13$ , and well 3:  $0.25\pm 0.01$ . In addition, an important variability was observed in the same well over time. Fluoride concentrations in three consecutive months were, for February:  $1.43\pm 0.03$ , March:  $1.45\pm 0.02$ , and April:  $0.95\pm 0.03$  with ANOVA  $p < 0.05$ . Fluoride concentration in groundwater has increased in the last 7 decades. The use of fertilizers and other agricultural technologies could be the cause of the increase. As a consequence, temporal sampling of different wells should be performed in order to determine fluoride concentration in each area.

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Keywords: Argentina; Drinking water; Fluoride changes in groundwater; Groundwater fluoride; Well F water changes.

## **PARTIAL FREEZING—A NEW APPROACH FOR FLUORIDE REMOVAL FROM WATER**

Drinking water provides many elements for the human body, one of them being fluorine, as fluoride ion, which has adverse health impacts in high concentrations, especially for babies and children. For this reason, the concentration of fluoride must be kept below the drinking water maximum standards. There are different processes for removal of fluoride from water such as coagulation, precipitation, adsorption, ion exchange, and membrane processes. In this study, a new approach for fluoride removal using a freezing process is reported. Drinking water samples with different concentrations of fluoride were prepared. Each water sample was put in a freezer and allowed to be partially frozen. The ice crystals were separated from the solutions. The liquid part was discarded, and the ice crystals were stored at room temperature to melt and then analyzed for fluoride. Findings indicated that partial freezing results in reduction of fluoride concentration down to the desired drinking water standards. By this method, about 20% of the initial volume of water will be produced with the low fluoride concentration. Since most households have a refrigerator, this method is very easily accessible and environmentally friendly compared to other treatment processes. Because, the quantity of drinking water consumed individually by infants and babies is about 1–2 L/day, preparation of that much water by partial freezing is relatively easy, and therefore can be a good way for small-scale fluoride removal from water in homes.

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Keywords: Drinking water; Fluoride removal; Freezing process; Water defluoridation.

## **EVALUATION OF GROUND WATER FLUORIDE CONCENTRATION IN HAMADAN PROVINCE, IRAN**

This study was carried out to determine the groundwater fluoride concentration in Hamadan province located in the western part of Iran. Groundwater samples were collected from 192 sampling points during dry and wet seasons. Fluoride concentration was determined in the samples using the SPADNS spectrophotometric method. Results showed that 49% of the fluoride concentrations in the samples are less than the recommended standard level. Using mouthwash and foods containing fluoride is recommended.

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Keywords: Fluoride in water; Groundwater; Hamadan province, Iran.

## **FLUORIDE ADSORPTION BY PUMICE FROM AQUEOUS SOLUTIONS**

Drinking water provides many vital elements for the human body, but the presence of some dissolved elements at higher than permissible concentrations can



endanger human health. Among the dissolved elements in drinking water, fluorine, as fluoride ion, is noteworthy because very low or very high concentrations are reported to have adverse health impacts, e.g., increased dental caries at very low concentrations, and dental fluorosis at high levels of intake. Therefore, the fluoride concentration should be kept at acceptable levels. In this study, pumice was used for fluoride removal from drinking water. It was found that the fluoride sorption kinetics fitted a pseudo-second-order model. The maximum sorption capacity of pumice was 13.51 mg/g at laboratory temperature (24°C) with maximum sorption occurring at pH 3. Results indicated that the fluoride sorption agreed with the Freundlich isotherm model. This study has shown that pumice is an effective, inexpensive adsorbent for fluoride removal from aqueous solutions.

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Keywords: Adsorption isotherm; Aqueous solution; Fluoride removal; Pumice.

### DEFLUORINATION OF WATER BY ACID-MODIFIED CLINOPTILOLITE

Clinoptilolite, a natural zeolite, was used as an adsorbent for fluoride removal from water. In order to enhance the clinoptilolite sorption capacity for fluoride, it was chemically modified using phosphoric acid and investigated in a series of batch experiments using fluoride concentrations in aqueous solution ranging from 2 to 10 mg/L. The influence of acid concentration, contact time, solution pH, initial concentration, and adsorbent mass was studied. The acid-modified clinoptilolite exhibited higher adsorption of fluoride than unmodified clinoptilolite. The results showed that pH in the range of 4 to 9 has a moderate effect on fluoride adsorption. Maximum absorption efficiency occurred with 0.7 molar phosphoric acid to modify the clinoptilolite. The adsorbed amount of fluoride also increased with the initial concentration of the metal ions, as expected. Among the models tested, the adsorption equilibrium for fluoride was best described by the Freundlich isotherm.

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Keywords: Acid modification; Adsorption; Aqueous solution; Clinoptilolite; Fluoride removal.

### FLUORIDE ADSORPTION FROM AQUEOUS SOLUTIONS USING BARLEY HUSKS AND BARLEY HUSK ASH

The ability of the barley husk and barley husk ash to remove fluoride from aqueous solution through adsorption has been investigated. Batch kinetics and isotherm studies were carried out under varying experimental conditions of contact time, fluoride concentration, adsorbent dose, and pH. Adsorption equilibrium of barley husk ash was reached within 4 hr for fluoride concentrations of 2–5 mg/L. Kinetics of adsorption obeyed a first-order rate equation. The

adsorption of fluoride increased with increasing the solution pH up to 7. The suitability of the Freundlich and Langmuir adsorption models for fitting the equilibrium data were investigated for each fluoride-sorbent system. The results showed that the equilibrium data for all the fluoride sorbent systems fitted the Freundlich model ( $R^2=0.95$ ) best within the concentration range studied. A comparative study showed that barley husk ash is more effective (87%) than barley husks (33.7%) for fluoride removal. We conclude that barley husk ash can be used as an efficient adsorbent material for removal of fluoride from water and wastewater.

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Keywords: Adsorption; Aqueous system; Barley husk; Barley husk ash; Fluoride removal.

### **ION CHROMATOGRAPHY METHOD FOR DETERMINATION OF INORGANIC ANIONS IN IRANIAN BOTTLED DRINKING WATERS**

Analysis of seven inorganic anions including fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate was performed on 10 brands of mineral bottled waters available in Iran using ion chromatography (IC). Two samples of each brand were selected from different times of production, one in autumn and another in winter 2010. The measured concentrations were then compared with the values on the labels by T-test. The means of difference between seasons and marks were investigated using two-way analysis of variance (ANOVA). The mean fluoride, chloride, nitrate, and sulfate ion levels of the samples were 0.16, 20.63, 3.05, and 24.43 mg/L, respectively, omitting the other three anions. Results of these determinations generally show a low content of anions in the bottled waters. All the samples were within the acceptable levels of the World Health Organization (WHO) guidelines and were lower than the maximum contaminant levels (MCL) established by the United States Environmental Protection Agency (USEPA). Comparison of label values with our measurements showed that 11 label values had marked differences from our findings. ANOVA of experimental results of different season samples revealed no difference in ion content, whereas analysis of the different brands indicated meaningful difference in the ion content of the samples.

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Keywords: Bottled water; Fluoride analysis; Ion chromatography.

## **PREVALENCE OF DENTAL FLUOROSIS AMONG PRIMARY SCHOOL CHILDREN IN RURAL AREAS OF INDIA**

Fluorosis is one of the common but major emerging areas of health research in the tropics. The Chandrapur district of Maharashtra State, which adjoins the Arabian Sea in the western part of south-central India, is an endemic fluorosis area. There clinical cases of dental fluorosis have been reported at a tertiary care teaching hospital in the field practice area of the Department of Community Medicine. Since dental fluorosis is a biomarker of exposure to fluoride, we assessed the prevalence and severity of dental fluorosis among primary school children in the service area. Children in six primary schools of three villages in the field practice area of the Rural Health Centre of Faculty of Medicine were surveyed. Every child was clinically examined at the school by calibrated examiners with Dean's fluorosis index recommended by the World Health Organization. Chi-square test, Chi-square trend test, and Spearman's rank correlation coefficient test were used for statistical analysis of 275 children of age of 5 to 12 (125 boys and 150 girls). The overall dental fluorosis prevalence was 31.4%. Dental fluorosis increased with age ( $P < 0.001$ ), whereas gender difference was not statistically significant. The community fluorosis index (CFI) score in the three villages was 0.43, 0.54, and 0.54 with 5.6%, 4.8%, and 1.4% of objectionable (moderate to severe) dental fluorosis. Correlation between water fluoride content and CFI values in four villages was noted to be significantly positive. Three out of six villages studied were in 'borderline' public health significance (CFI score 0.4-0.6). A well-designed epidemiological investigation should be undertaken to evaluate the risk factors associated with the conditions in the study region.

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Keywords: Chandrapur district; Community fluorosis index; Dental fluorosis; Maharashtra, India; Wardha district; Water fluoride level.

## **A CASE STUDY OF DENTAL FLUOROSIS PREVALENCE IN RURAL COMMUNITIES IN NORTHWEST IRAN**

In this study, the prevalence of dental fluorosis and its relation to the fluoride content of drinking water in rural communities of East Azerbaijan province in Iran was studied. Three villages were selected representing low, medium, and high exposure to fluoride: Bashesizkooch in Bostanabad (fluoride concentration = 0.12 mg/L), Nagharehkub in Ahar (0.6 mg/L new water source; 1.1-1.2 mg/L old source), and Gharehbolaq in Jolfa (0.35 mg/L new water source; 2.4 mg/L old source). The previous history and new analyses of the supplies were used to determine the quality of the water sources. All residents older than age 6 were examined. Dental fluorosis was observed in 62.7% of individuals examined. Grade 1 fluorosis was diagnosed in 31.5% of the participants, grade 2 in 22.4%, grade 3 in 7.7%, and grade 4 in 1.1%. There was a significant difference between the

villages in the prevalence of fluorosis ( $p < 0.001$ ): Bashsizkooch (32.5%), Nagharehkub (70.5%), and Gharebolaq (83.3%). Fluorosis was observed in both permanent and temporary teeth. The mean cumulative fluoride index in people with and without fluorosis was 22660.2 and 4743.2, a ratio of 4.77:1. There was a correlation between the index and fluorosis ( $r = 0.413$ ). Responsible authorities are requested to do all they can to curtail excessive fluoride intake from drinking water.

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### **FLUORIDE LEVELS IN DRINKING WATER OF EAST AZERBAIJAN PROVINCE, IRAN**

For the present study in the East Azerbaijan province of Iran, results of analyses of 668 samples of urban and rural water sources in 19 counties, obtained from the Province Health Center, were checked, and the amount of fluoride, hardness, pH, alkalinity, and electrical conductivity tabulated. After statistical analysis the data were compared with standards. In the case of villages with a high level of fluoride, new samples were drawn and analyzed. Electrical conductivity of these drinking waters ranged from 104 to 4660  $\mu\text{s}/\text{cm}$  with an average of  $740 \pm 570$   $\mu\text{s}/\text{cm}$ . Hardness ranged from 40 to 1434 mg/L as  $\text{CaCO}_3$  with an average of  $228 \pm 100$  mg/L. In 91.3% of drinking water sources, the fluoride content was  $\leq 0.5$  mg/L, in 22 sources, it was  $> 1$  mg/L and led to dental fluorosis. Consumption of hard water or of water with a high content of solids should be limited as much as possible. For water sources with a fluoride content above 1 mg/L, the possibility of providing a new source should be considered.

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### **SUPPLEMENTS, ISOTONIC DRINKS, AND ENERGY DRINKS AS POTENTIAL SOURCES OF DIETARY EXPOSURE TO FLUORINE COMPOUNDS IN ATHLETES**

Supplements and isotonic drinks are major components of the diet of athletes, whereas energy drinks are commonly used by nearly the entire population. The use of animal proteins and plant-origin substances in these products suggests that they may also contain fluorine compounds. As most supplements are ingested as aqueous solutions, they may constitute a source of easily absorbable fluoride. This potential exposure may be important in balancing the diet and predicting potential adverse effects. The present study was performed to determine the role of the most popular protein and creatine supplements and isotonic drinks and energy drinks

that are available on the Polish market as potential sources of exposure to dietary fluorine compounds, not usually disclosed by the manufacturer. Measurements of fluoride concentrations in individual samples were carried out using an Orion (USA) fluoride ion-selective electrode. Statistical analysis of the results was based on non-parametric tests (Friedman ANOVA test and Mann-Whitney U test). Differences were considered statistically significant for  $p < 0.05$ . The study was supplemented by the results of a survey of the amount, type, and frequency of consumption conducted among consumers of these products. Fluoride was found in all supplements, isotonic drinks, and energy drinks. The highest fluoride levels were detected in creatine-containing products, while the lowest levels were found in energy drinks. Fluoride concentrations in creatine supplements were higher, in a statistically significant manner, than in protein supplements ( $p < 0.05$ ), energy drinks ( $p < 0.05$ ) and isotonic drinks ( $p < 0.02$ ). A statistically significant difference in fluoride levels was observed between protein supplements and energy drinks ( $p < 0.02$ ). No significant differences were identified between fluoride levels in protein supplements and isotonic drinks as well as between energy drinks and isotonic drinks. Creatine supplements, with the highest levels of fluoride, are also the most frequently used supplements in this group of products. Considering the frequency and long-term use of supplements, individuals extensively engaged in sports should also keep in mind their potential exposure to fluorine compounds from these sources. The physical exercise-related burden to bones, joints, and ligaments may be intensified due to incorporation of fluoride into hydroxyapatite structures, leading to increased risk of injuries and diseases of the bone-articular system.

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Keywords: Diet supplements; Isotonic drinks; Fluorine compounds.

## **FLUORIDE REMOVAL FROM WATER BY NANOFILTERS**

Fluoride in high concentration in drinking water can cause toxic effects to different parts of the human body, especially teeth and bones. According to the WHO guideline, the fluoride concentration in drinking water should be limited to 1.5 mg/L. Therefore, finding a suitable method to remove the excess amount of fluoride is essential. Today, economic and environmental aspects are important factors for the selection of an effective technology for water treatment such as reverse osmosis (RO) with improved membrane technology. In the present study, the efficiency of the nanofiltration membrane (NF33, 1812-50, FILMTEC) to remove fluoride from water by RO was investigated, taking into account the initial amount of fluoride, sulphate, chloride, and other anions, along various cations, electrical conductivity (EC), and physical parameters such as flow rate. The results showed that, in all experiments, fluoride concentrations in the permeate water were reduced to the standard range. However, fluoride removal efficiency

decreased with increasing initial levels of fluoride, sulphate, and EC in the feed water, and with increasing flow rate. It was also found that removal efficiency was higher for CaF<sub>2</sub> than for NaF.

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Keywords: Fluoride removal; Membrane technology; Nanofiltration; Reverse osmosis; Water treatment.

### **FLUORIDE CONCENTRATION IN THE DRINKING WATER SUPPLY SOURCES AND DISTRIBUTION NETWORK OF QOM, IRAN**

This study was undertaken to measure the concentration of fluoride in drinking water supplied by the distribution network in Qom City in Northcentral, Iran, and to compare it with the level of total dissolved solids (TDS) and electrical conductivity (EC). All sources of drinking water, the urban and nearby rural, were studied. Urban water sources included the urban distribution network, Ali Abad station, and the desalination system. For F analyses, the standard SPADNS method was used with a DR/4000s Spectrophotometer (HACH Company). The results showed that the mean fluoride concentrations in rural areas, whose water is mainly provided by groundwater sources, was in the range 0.05–0.903 mg/L. In the Urban distribution network it was 0.825 mg/L, in Ali Abad station 0.115 mg/L, and in the desalination system 0.2411 mg/L. Due to the hot climate of Qom, the average fluoride concentration in all sources, except in some of the groundwater sources and urban distribution networks, is less than the permissible concentration set by the Iranian standards. With the exception of some of the groundwater sources and urban distribution network, it is also less than the permissible concentration proposed by WHO. It seems that in most of the drinking water sources the average fluoride level is less than ideal for the prevention of dental caries. The highest level of fluoride (2.18 mg/L) was found in the Neyzar rural area.

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### **HEALTH EFFECTS OF HIGH FLUORIDE IN GROUNDWATER IN PARTS OF TWO DISTRICTS IN CENTRAL INDIA**

Drinking water containing high concentrations of fluoride is harmful to human health. To take adequate preventive measures, it is essential to know the spatio-temporal distribution of F in groundwater. This study was conducted to determine the fluoride content in groundwater and its relationship to the prevalence of fluorosis in the Yavatmal and Chandrapur districts of the Vidarbha Region of Central India. Nearly 18% of the sampled wells have fluoride concentrations above the desirable limit ( $\leq 1.0$  mg/L), the highest value being 8.8 mg/L. High

fluoride concentrations occur primarily in regions of Yavatmal and Chandrapur. Dental fluorosis was observed in five villages in Chandrapur district and in two villages in Yavatmal district, whereas skeletal fluorosis was found in two villages of Chandrapur district. Among the 416 subjects surveyed 41 (17.86%) and 23 (16.33%) subjects had signs and symptoms of either dental or skeletal or both types of fluorosis. The spatial distribution of fluoride in groundwater, as indicated by hydrogeochemical analyses, correlated well with the prevalence of dental and skeletal fluorosis. Because the region falls in an industrial coal-mining belt, a large fraction of the population is at risk for fluorosis. Utilization of water from phreatic aquifers will minimize the incidence of fluorosis in the endemic areas from deep wells that are discharging fluoride-contaminated groundwater. To cope with these problems, a multi-sectoral approach is needed that includes broad community participation, installation of appropriate types of pumps, and new, less-polluting irrigation methods.

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Keywords: Groundwater; Health-risk; High fluoride in groundwater; Preventive measures.

### **DRINKING WATER FLUORIDE CONCENTRATION AND ITS RELATIONSHIP WITH DMFT INDEX IN MIANEH CITY, IRAN**

The purpose of this research was to determine the fluoride concentration in drinking water of Mianeh city in the province of East Azerbaijan, Iran. This cross-sectional research was carried out on all 14 groundwater sources of drinking water in 2008. The results show that the mean fluoride concentration in spring, summer, autumn, and winter was  $0.295 \pm 0.039$ ,  $0.47 \pm 0.17$ ,  $0.48 \pm 0.18$ , and  $0.4 \pm 0.06$  mg/L, respectively. Moreover, the concentration of fluoride in all water sources was below the permissible concentration based on Iran drinking water standards, but it was in standard range proposed by WHO. In addition, the DMFT index of the children between 6 and 9 years old in Mianeh was higher than the Iran DMFT index.

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Keywords: Dental caries; Dissolved elements; Drinking water; Fluoride concentration; Iran; Mianeh city, Iran.

### **HUMAN SKELETAL FLUOROSIS IN INDIA**

Fluoride is a major health concern in India, especially because of the warm climate and relatively high levels of fluoride in many drinking water sources. With excessive intake, fluoride can have serious toxic effects that are manifested in various forms and severity in the form of fluorosis. In the body, fluoride toxicity is

seen in disturbances of various hard tissues, including teeth and bones along with osteophytosis and thickening and calcification of ligaments. In our studies we have observed these conditions and have detected radiological changes in teeth and bones that indicate fluorosis. We have also analysed water samples in areas of fluorosis and found levels that are higher than recommended limits.

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Keywords: Bones; Fluorosis.

### **EFFECT OF AREA-SPECIFIC MINERAL MIXTURE SUPPLEMENTATION ON CLINICO-HAEMATOLOGICAL AND MINERAL PROFILE OF FLUOROTIC BUFFALOES**

Supplementation with an area-specific mineral mixture at 50 g per animal per day for 60 days to fluorotic buffaloes (group I) resulted in a decline in plasma concentrations of Ca, Mg, Mn, Zn, and Mo along with increased Cu, Fe, and F on day 60. On the other hand, supplementation of the same mineral mixture to nonfluorotic buffaloes (group II) resulted in increase in plasma Ca, Cu, Fe, Mo, and F, but a decrease in Mg, Mn, and Zn in blood plasma on day 60. Day 30 values, however, showed variable trends in both group I and group II. The peripheral blood picture in both groups revealed an increase in hemoglobin, hematocrit, and total erythrocyte count on both day 30 and day 60. Overall, the results showed a favourable response from mineral mixture supplementation in fluorotic buffaloes on the blood mineral profile. Moreover, improvement in blood picture, increase in milk production, and clinical recovery-from lameness further indicated beneficial effects of area-specific mineral mixture supplementation in these fluorotic buffaloes.

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Keywords: Blood; Fluoride toxicity; Haematology; Mineral mixture; Minerals.

### **ALTERATIONS IN BLOOD CONCENTRATIONS OF MACRO- AND MICRO-MINERALS IN WATER BUFFALOES LIVING IN ENDEMIC FLUOROSIS AREAS OF PUNJAB**

Levels of certain micro and macro minerals were estimated in water buffaloes affected by clinical fluorosis while they were reared in four endemic fluorosis districts in Punjab state, India. Water fluoride concentration was highest in the district of Bathinda (mean level 11.66 ppm), followed by Sangrur (mean level 3.13 ppm), Mansa (mean level 3.06 ppm), and Ferozepur (mean level 1.82 ppm). Mean plasma fluoride level in fluorotic water buffaloes was almost double that observed in healthy control animals. Decline in plasma phosphorus, copper, manganese, molybdenum, cadmium, chromium, nickel and lead, along with non-significant



fluctuations in plasma calcium, magnesium, zinc, iron, sodium, potassium, arsenic, cobalt, mercury and sulphur were observed. Results of the study suggested that excess fluoride intake alters the concentration of different macro- and micro-minerals in blood. Further research is required to elucidate possible mechanisms behind this observation and to evaluate the overall effects of these changes in the pathogenesis of fluorosis.

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Keywords: Buffalo; Minerals; Toxicity.

### **CHANGES IN CORTISOL, OXIDATIVE STRESS INDICES, AND SERUM BIOCHEMISTRY IN FLUORIDE-INTOXICATED RABBITS**

This study was undertaken to evaluate changes in the plasma of cortisol levels, oxidative stress indices, and biochemical parameters in liver and kidney functions of fluoride-intoxicated rabbits. Twenty-four New Zealand White rabbits, 4 to 6 weeks old, weighing between 600 and 800 g, were divided into four equal groups of 6 animals each. Drinking water containing 50 (Gr. I), 100 (Gr. II), 200 (Gr. III), and 0 (Gr. IV, Control) mg NaF/L was provided *ad libitum* for a period of 90 days. Blood samples were collected on day 0, 45, and 90 by cardiac puncture using heparinized sterile syringes, followed by separation of the plasma. A significant increase in plasma cortisol level was observed in all the fluoride treated groups on day 90 that exhibited a high positive correlation ( $P = 0.948$ ) with plasma fluoride concentration. An increase in lipid peroxide levels and a decrease in superoxide dismutase and catalase activities were observed in Group II and III on day 90. However, changes in these oxidative stress parameters did not show any consistent relationship with the dose level of fluoride exposure. An increase in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities, along with blood urea nitrogen and creatinine levels, indicated hepatotoxic and nephrotoxic effects of fluoride intake in the rabbits, which were more marked on day 90 in the Group II and III animals.

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Keywords: Cortisol; Fluorosis; Kidney; Liver; Oxidative stress; Rabbits.

### **INFLUENCE OF FLUORIDE ON COCOON PRODUCTION/ PROTEIN PROFILES IN DIFFERENT IMPROVED SILKWORM VARIETIES, *BOMBYX MORI* L.**

Crop losses in sericulture are directly related to unfavorable and/or highly variable environmental conditions along with poor quality mulberry leaves, which depend on the location of the source and the state of environmental factors such as soil, water, and air. Fluoride at higher concentrations acts as a cumulative poison and causes adverse effects on mulberry silkworm larvae, in both physiological and

economic aspects, in India and China. In India, besides the impact of advanced rearing technologies, the quality of the cocoon, as elsewhere, depends mainly on healthy larval growth, which in turn reflects the qualitative and quantitative nutritional content of the mulberry leaves fed during silkworm rearing. In the present study, the commercial characteristics of the cocoon such as length, width, weight, shell weight, shell ratio, filament length, and filament weight significantly decreased on exposure to sublethal (5.5 mg/kg bw) and sub-sublethal doses (2.75 mg/kg bw) of fluoride in the mulberry leaves compared to controls. However, the level of decrease was more pronounced during sub-lethal intoxication and marginal at sub sub-lethal dosages under fluoride stress. In our protein metabolic profile studies, we observed a significant decrease in total proteins and a significant increase in the levels of the free amino acids, the activities of protease, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, and the amount of ammonia. However, the increase in the urea content was not significant. The variation in the protein metabolic profiles indicates an altered cellular metabolism thereby leading to lower quality cocoon production. Furthermore, we observed that the influence of fluoride on cocoon production is significantly greater in recently improved silkworm varieties. Thus the occurrence of fluoride contamination in the Indian peninsular region exhibits a measurable negative influence on the sericulture industry.

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Keywords: *Bombyx mori* L; Cocoon growth; Fluoride in mulberry leaves; Sericulture in India; Silkworms.

### **FLUORIDE BIOACCUMULATION AND ELECTRON MICROSCOPY STUDY OF THE FLUOROTIC SILKWORM MIDGUT**

The present study examined the magnitude and relationships of the bioaccumulation and histopathological changes in midgut tissue of silkworms treated with sub-sublethal (2.75 mg/kg bw) doses of fluoride in their mulberry leaf diet, since the mulberry gardens occasionally get contaminated with fluoride. An electron microscopy study was made on midgut histopathology of silkworms exposed to fluoride. The midgut of the silkworm on exposure to the sub-sublethal dose of fluoride exhibited little regression in the columnar epithelial cells. The nuclei were clearly evident in the apical region of columnar and goblet cells. Spread of cytoplasmic granules and globules from the columnar cells into the space lying in between the peritrophic membrane and epithelium was observed on the 2<sup>nd</sup> day after exposure of fluoride. On the 3<sup>rd</sup> day the columnar cells of the anterior midgut were swollen apically and began to extrude large cytoplasmic vesicles into the gut lumen. The goblet cells were vacuolated, the membranous vesicles were absent, but the regenerative cells remained unaffected. However, on the 4<sup>th</sup> day of exposure to the sub-sublethal dose of fluoride, there were no significant conspicuous changes. The goblet and columnar cells discharged membranous vesicles, thus indicating normal functioning of the midgut after due

recovery from the histopathological injury. Bioaccumulation of fluoride showed an increment at the 2<sup>nd</sup> and 3<sup>rd</sup> days, whereas the increment was not statistically significant at the 4<sup>th</sup> day of sub-sublethal doses of exposure. Thus, variations in the fluoride concentrations in the diet of silkworms indicate toxic effects on cellular metabolism leading to impairment of protein synthetic machinery as revealed by histopathological studies.

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Keywords: Fluoride bioaccumulation; Midgut histopathology; Silkworm midgut.

### **SILKWORM GROWTH AND NUTRITION—IMPACT OF FLUORIDE TOXICITY**

Mulberry as a foliage crop is grown extensively in the states of Andhra Pradesh, Karnataka, and Tamil Nadu, India, for feeding silkworms. The mulberry gardens in these regions occasionally get contaminated with many kinds of pollutants, and problems have cropped up in sericulture due to intoxication of silkworms feeding on contaminated mulberry leaves. Among various environmental polluting factors, fluoride exerts a very profound influence on the growth of the silkworms, especially where the fluoride concentrations range between 2 and 25 ppm in ground waters in drought-prone areas of India. Fluoride is generally regarded as highly toxic to all organisms. Fluoride ingestion by silkworms has been found to result in a significant loss of silkworm body weight and a decrease in faecal output, which apparently is due to decreased consumption of food. The changes in the nutritional parameters appear to be dose and time dependent in both sublethal (5.5 mg F/kg bw) and sub-sublethal (2.75 mg F/kg bw) dosages of fluoride consumed by silkworms. However, the changes in the sub-sublethal level of fluoride ingested by silkworms on the 4<sup>th</sup> day were less compared to sublethal dose-treated silkworms, indicating that the silkworms on prolonged exposure can recover from the toxic effect of fluoride at a lower dose.

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Keywords: Body weight; Faecal output; Fluoride effect on silkworms; Mulberry; Silkworms and fluoride.

### **MITIGATING EFFECTS OF TRIPHALA ON NON-ENDOCRINE FLUORIDE TOXICITY**

Healthy adult female rats (*Rattus norvegicus*) were administered 10 mg NaF/kg bw/day and 30 mg triphala (a popular Ayurvedic herbal product)/kg bw/day orally. Both doses were given individually and also in combination for 30 days to investigate the non-endocrine organ fluoride toxicity in muscle and blood with reference to energy metabolism and its reversal by triphala. Fluoride caused an alteration in body and organ weights and decreased total protein levels in both gastrocnemius muscle and serum of the NaF-treated rats. The treatment also resulted in a reduction in phosphorylase and succinate dehydrogenase (SDH)

activities along with an increase in glycogen levels probably affecting energy and carbohydrate metabolism. This interpretation is further supported by increased fluoride levels in the muscle tissue. The SGPT and SGOT levels were elevated by fluoride treatment indicating impairment of liver function. Fluoride also brought about a significant decline in the hemoglobin level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin count (MCHC) in the red blood cells, indicating anemia and probable adverse effects on the haemopoetic system. A significant increase in erythrocyte sedimentation rate (ESR) suggested overall toxic effects in the body. A significant increase in the white blood cell count also indicated toxicity from NaF to the immune system. Co-administration of triphala to the fluoride-treated rats resulted in partial reversal of the ill effects in muscle and blood and on body weight.

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Keywords: Blood; Fluoride intoxication in rats; Muscle weakness; Non-endocrine effects; Phosphorylase; Rat study; Triphala.

### **ROLE OF TRIPHALA ON FLUORIDE NEUROTOXICITY IN THE RAT**

To investigate effects of sodium fluoride (NaF) on the cerebellum cerebral hemisphere brain of rats and its possible amelioration by the popular Ayurvedic herbal health product triphala, adult female Wistar rats (*Rattus norvegicus*) were administered 10 mg NaF/kg bw/day and 30 mg triphala/kg bw/day for 30 days. A significant decline in body and brain tissue weights after NaF treatment was noted. The decreased succinate dehydrogenase (SDH) activity indicated an alteration in mitochondrial structure and function by fluoride poisoning affecting energy metabolism. An increase in the total lipid could be the result of the inhibition of enzymes and reduction of fatty acid synthesis. Moreover, increased lipid peroxidation indicated an oxidative stress in the brain. These effects were correlated with histological changes and accumulation of fluoride in the neural tissue. However, these changes were ameliorated by co-supplementation of triphala with NaF. The antioxidant properties of the herbal product triphala therefore had an alleviating effect on NaF-induced neurotoxicity in rats.

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Keywords: Antioxidants; Brain parts; Neurotoxicity; Oxidative stress; Rat study; Triphala.

### **TRIPHALA, AN EXCELLENT ANTIOXIDANT FOR MITIGATION OF FLUORIDE ENDOCRINE TOXICITY**

Sodium fluoride (NaF) at a dose of 10 mg/kg bw was administered orally to female rats daily for 30 days to evaluate its affect on thyroid and ovarian functions in relation to oxidative stress in rats. The parameters studied were gravimetric and biochemical indices in endocrine tissue of fluoride-fed rats with histopathology.

Treatment brought about an alteration in body and organ weights and in various biochemical indices like total proteins and succinate dehydrogenase (SDH) levels thus affecting the energy status of these organs. Lipid peroxidation (LPO) increased compared to controls. Thyroid hormone levels and TSH were also affected, and histomorphology exhibited alterations. Supplementation with the Ayurvedic herbal antioxidant triphala (30 mg/kg bw/day over the 30-day experiment revealed that the toxic effects of fluoride on endocrine organ functions were essentially abolished, thereby showing that triphala is able to mitigate NaF-induced endocrine toxicity in a rat model.

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Keywords: Antioxidants; Endocrine organs; Fluoride in rat study; Sodium fluoride given to rats; Thyroid hormones; Triphala.

### **APPLICATION OF MODIFIED WHEAT STRAW FOR FLUORIDE REDUCTION FROM AQUEOUS SOLUTIONS: ISOTHERMS AND KINETICS**

High levels of fluoride in drinking waters due to anthropogenic and natural sources are a major human health risk in some communities around the world. Different methods have been suggested for fluoride reduction in drinking water. Among them, adsorption methods have been widely applied with efficient results. Attempts to find more cost-effective and efficient adsorbents are continuing. The aim of this work was to determine the feasibility of modified wheat straw together with sodium bicarbonate for water defluoridation. In a batch-scale study in the laboratory, parameters including pH, contact time, initial fluoride concentrations, and adsorbent dosages were investigated. The kinetics and equilibrium isotherms were also examined. Adsorption was influenced by the initial fluoride concentration, adsorbent dosage, and pH. Among the models tested, the Langmuir and Freundlich models gave the best fit. Desorption experiments show that fluoride ions are strongly bound to the adsorbent and exhibit low rate of desorption. We conclude that adsorption by modified wheat straw is an efficient and reliable method for reduction of elevated fluoride levels in water.

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Keywords: Adsorption; Adsorption isotherm; Fluoride in water; Sodium bicarbonate; Wheat straw.

### **EFFECT OF SODIUM FLUORIDE ON CATALASE ACTIVITY IN THP-1 MACROPHAGES**

Reactive oxygen species (ROS) generated by macrophages are of prime importance for inflammatory response in humans. In this study, the inhibitory effect of sodium fluoride (NaF) on macrophage catalase (Cat), which normally degrades ROS, was examined over a range of NaF concentrations and time. Macrophages obtained from the THP-1 monocytic cell line were incubated with 0.5 mM, 1 mM, and 5 mM NaF over 10, 30, and 60 min of incubation. Catalase activity was estimated spectrophotometrically, and the protein concentration was measured by the Bradford method. Data were checked with ANOVA Friedman and Wilcoxon matched-pair tests with  $p < 0.05$  being considered significant. NaF exerted a statistically significant inhibitory effect on the catalase activity over a range of concentration and time ( $p < 0.05$ ). Inhibition of catalase activity by NaF used at the concentration of 0.5 mM was significant after 30 and 60 min ( $p < 0.05$ ;  $n = 6$ ) compared to controls. Addition of 1 and 5 mM NaF significantly reduced enzyme activity after 10 min ( $p < 0.05$ ) as well as after 30 and 60 min. Thus, NaF impaired antioxidant systems and reduced cell protection against ROS.

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Keywords: Antioxidant enzymes; Catalase; Fluoride and catalase; THP-1 macrophages.

### **FLUORIDE LEVEL IN 18 BRANDS OF BOTTLED DRINKING WATER AVAILABLE IN IRAN**

With increased consumption of bottled drinking water around the world, monitoring the biological and chemical quality of the water is essential. The purpose of this study was to determine fluoride level of 18 bottled drinking water brands in Iran market to compare it with standard values and values written in the label and also to compare the EC (electrical conductivity) with the fluoride level. In June 2012, 18 brands of bottled water common on the Iran market were purchased and the fluoride concentration of each sample was measured three times by the SPADNS method using a spectrophotometer DR/4000 (HACH, USA). The EC was measured by a digital EC meter (HANNA, ITALY). The mean fluoride level was 0.23 mg/L with a range of 0.09–0.39 mg/L. In 69.2% of the samples, the values written on the label were lower than measured values but were much higher in 30.8% of the labels. The correlation coefficient between fluoride level and EC was  $r = 0.27$ ) indicating no significant relation between these two variables. Fluoride levels in all samples were lower than the national permissible values, and in some samples were lower than standard recommended values.

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Keywords: Bottled drinking water; Electrical conductivity; Fluoride in water; Iran; Potable water.

## DEFLUORIDATION OF AQUEOUS SOLUTIONS WITH WASTE ALUMINUM FILINGS

Anthropogenic activities involving the fertilizer, aluminum, steel, ceramics, bricks, glass, and nuclear energy industries play an important role in water pollution by fluoride (F). Defluoridation of industrial wastewaters by adsorption is one of the most favored methods, especially when performed with low-cost and locally available materials. Waste aluminum filings were studied in batch experiments in relation to pH (2–12), adsorbent dose (1–20 g/L), contact time (15–60 min), interfering anions (200 and 400 mg/L), adsorbent activation temperature (500–900°C), adsorption temperature (20–50°C) and initial F concentration (5–20 mg/L) were studied. The removal efficiencies increased with contact time, adsorbent dose, adsorbent activation temperature, pH, adsorption temperature, and initial F concentration. The efficiency of F removal in the presence of interfering anions decreased in the following order:  $\text{PO}_4^{3-} > \text{HCO}_3^- > \text{CO}_3^{2-} > \text{Cl}^- > \text{SO}_4^{2-} \geq \text{NO}_3^-$ . The adsorption was found to be endothermic and was better fitted for the Freundlich model, which is indicative of multilayer adsorption. Because aluminum filings can be used in column adsorption systems, their applicability appears to be more suitable than the use of alum-based methods.

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Keywords: Adsorption; Aluminum filings; Waste water defluoridation.

## EFFICIENCY OF GRANULAR FERRIC HYDROXIDE (GFH) FOR FLUORIDE REMOVAL FROM WATER

Granular ferric hydroxide (GFH), a combination of poorly crystallized FeOOH, was investigated for removal of fluoride (F) from water. Adsorption was studied in batch experiments at room temperature along with the effect of contact time, pH, initial F concentration, and  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  as interfering anions. Lower pH with higher initial F concentration and longer contact time increased the F removal efficiency. In the absence of interfering anions, maximum removal of F approaching 88% was achieved at pH 6 after 30 min from water containing 7 mg F/L. Considering economic and feasibility aspects, adsorption of F from water with GFH is recommended, especially at wastewater plants.

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Keywords: Fluoride removal; Granular ferric hydroxide; Wastewater treatment; Water defluoridation.

## REGULATION AND REVERSAL OF EFFECTS OF FLUORIDE ON CALCIUM HOMEOSTASIS IN RATS

This study was undertaken to determine the effects of fluoride on calcium homeostasis and gene expression together with their reversal by normal calcium and fluoride free water (FFW). In phase I lasting 6 months, 76 weanling, male rats were divided into equal four groups that received a normal calcium (0.5%) diet (NCD) + FFW (Group 1/control n = 12), a low calcium (0.25%) diet (LCD) + FFW (Group 2, n = 16), NCD + 100 ppm fluoride (Group 3, n = 24, NCD + F), and LCD + F (Group 4, n = 24). After 6 months, 6 animals from group 1 and 8 animals from all other groups were sacrificed by CO<sub>2</sub> asphyxiation. In phase II (reversal of phase I), lasting for 3 months, LCD of group 2 and 4 was replaced by NCD. In phase II, group 3 and 4 were divided into two subgroups (n = 8), i.e., 3a, 3b and 4a, 4b, respectively. Subgroups 3a and 4a received FFW whereas 3b and 4b were continued with fluoride water. Blood was collected by retro-orbital sinus puncture after overnight fasting at the interval of 90 days and serum was separated and stored at –80°C. At end of phase I and II, the remaining rats were sacrificed by CO<sub>2</sub> asphyxiation. Proximal small intestine (5 cm) was collected and immediately washed with cold saline. Duodenal mucosa was scraped off, snap frozen, and preserved at –80°C for gene expression studies. Serum calcium (SCa) was measured by atomic absorption spectroscopy and serum 25-(OH) vitamin D<sub>3</sub>, 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> and intact parathyroid hormone were assessed using kits. Quantitative PCR was conducted for calcium sensing receptor (Casr), vitamin D receptor (Vdr), and Calbindin D 9K (Calb3). In phase I, SCa in groups 2, 3, and 4 was significantly lower than in group 1. In phase II (reversal phase), in spite of improvement in average SCa level, all experimental groups showed significantly lower SCa as compared to group 1. At day 90, serum PTH level in group 2 was higher than in group 1. As expected, LCD groups 2 and 4 had significantly lower serum 25-(OH) vitamin D<sub>3</sub> levels and significantly higher serum 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> as compared to NCD groups 1 and 3 in phase I. In phase II, subgroup 4b showed significantly higher PTH as compared to subgroup 3b. Serum 25-(OH) vitamin D<sub>3</sub> and serum 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> in all groups were comparable. Down regulation of any of the receptors studied was not observed in the presence of fluoride in phase I. However, in phase II, Calb3 expression of fluoride-treated subgroups 3b was down regulated 2.5 fold and 2 fold in 4b compared to subgroup 3b. It is concluded that adverse effects of chronic fluoride intoxication on calcium homeostasis are more pronounced with calcium deficiency. However, these effects may be reversible with adequate calcium nutrition and safe drinking water.



Chronic fluoride toxicity may affect calcium absorption in the duodenum due to an inhibitory effect on expression of Calb3—a key component for calcium absorption.

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Keywords: Calbindin D 9k; Calcium homeostasis; Calcium sensing receptor; Fluoride in rats; Vitamin D receptor.

## **MID-TERM OVERVIEW OF JAPAN INTERNATIONAL COOPERATION AGENCY (JICA) FLUOROSIS MITIGATION PROJECT PHASE 2 (2011) IN SOUTHERN INDIA**

### **1. THE REPORT OF THE WATER ANALYSIS FOR FLUORIDE AND TRACE ELEMENTS IN KRISHNAGIRI AND DHARMAPURI DISTRICTS, INDIA**

High fluoride concentration in groundwater is a critical toxicological issue in Krishnagiri and Dharumapuri in the State of Tamil Nadu, India. In 2008, the Japan International Cooperation Agency started a study entitled “Hogenakkal Water Supply and Fluorosis Mitigation Project.” This survey was performed to assess the fluoride and trace element levels in drinking water in the areas at the mid-term of this project. Seven water samples were collected and analyzed. Only one water sample exceeded the WHO guideline for fluoride concentration. No trace element exceeded the WHO guideline values. However, it is necessary to assess the effect of trace elements, for which WHO does not provide guideline values. Under the Hogenakkal Water Supply and Fluorosis Mitigation Project, the equipment needed to measure fluoride, such as fluoride ion meters, is now available and currently in operation. However, quality control, in general, must be improved in order to increase data accuracy. Also, accurate diagnosis of fluorosis is necessary to assess the effectiveness of this project.

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Keywords: Fluoride analysis; Fluorosis in India; Japan International Cooperation Agency; Trace elements.

## **FLUORIDE CONCENTRATION IN INFANTS' AND CHILDREN'S DRINKS IN POLAND**

Delivered at high doses and/or over long periods, fluorine compounds may negatively affect the function of the immune and nervous systems, cause allergies, and negatively alter thyroid function. Because of their higher susceptibility to fluoride absorption, children are at higher risk than adults for these consequences manifested by chronic fatigue, lowered IQ scores, and learning difficulties. Owing to the fact that drinks are an important element of children's diets, and that they are a source of fluoride more easily absorbed by the system, the intake of these drinks

should be taken into account when estimating the daily dose of fluoride delivered to the body. Particular types of drinks for children and infants may differ in terms of fluoride concentration, depending mostly on the species and origin of fruit and vegetables and the quality and quantity of water used in production of these drinks. The aim of this study was to determine the fluoride levels in the most popular drinks for children and infants as available on the Polish market. The products were divided into seven categories: fruit juices for children 3 months of age and above, puree-based juices, fruit drinks for kindergarten and school-age children, vitamin- and microelement-enriched drinks, milk-based drinks, and “one-day” juices. Measurements of fluoride concentrations were conducted using an Orion (USA) fluoride ion-selective electrode. Statistical analysis of the results was based on non-parametric tests (Friedman ANOVA test and Mann-Whitney U test). Differences were considered statistically significant for  $p < 0.05$ . The study was supplemented by the results of a survey conducted among parents and also considered the type and quantities of products offered to their children. The highest concentration of fluoride was determined in milk-based drinks, while the lowest was in fruit juices for children above 3 years of age and juices for infants above 3 months of age. The fluoride levels in milk-based drinks were higher than in fruit-based drinks ( $p < 0.01$ ), vitamin-enriched drinks ( $p < 0.05$ ), juices for infants above 3 months of age ( $p < 0.01$ ), puree-based juices ( $p < 0.01$ ) and “one-day” juices ( $p < 0.01$ ). Statistically significant differences in fluoride levels were also observed between vitamin-enriched drinks and juices for infants above 3 months of age ( $p < 0.01$ ) or fruit drinks ( $p < 0.01$ ). Milk-based drinks had the highest levels of fluoride. At the same time, such drinks are considered the healthiest. Knowledge of the content of fluorine compounds in drinks for children allows an informed choice of drinks offered to children to reduce the risk of the negative effects of fluoride on their systems.

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Keywords: Children’s drinks; Fluoride in drinks; Fruit juices; Infant drinks; Milk-based drinks; Vitamin- and microelement-enriched drinks.

## **EFFECT OF SODIUM FLUORIDE ON CATALASE AND GUAIACOL PEROXIDASE ACTIVITY IN SELECTED PLANT SPECIES**

Fluoride can directly induce generation of reactive oxygen species (ROS) and oxidative stress in both plants and animals. It inhibits or activates enzymes that protect organisms against imbalance between ROS and antioxidants. This study was conducted to determine the effect of sodium fluoride at a level of 190 mg F/L in Hoagland’s hydroponic culture medium on catalase (CAT) and guaiacol peroxidase (POX) activity in 14-day seedlings of nine plant species: bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), broad bean (*Vicia faba*), agrimony (*Agrimonia eupatoria*), white mustard (*Sinapsis alba*), wheat (*Triticum aestivum*), oat (*Avena sativa*), millet (*Panicum miliaceum*), and corn (*Zea mays*). For each

plant species a control experiment without NaF treatment was conducted. Enzyme activities were measured spectrophotometrically by standard procedures using a UV-1800 Shimadzu spectrophotometer. Catalase activity increased significantly in bean, pea, agrimony, oat, and millet but decreased in corn and broad bean tissues compared to control plants. Activity of peroxidase was significantly higher in treated plant tissues than in control plants in the case of wheat, oat and agrimony, whereas in broad bean, millet, and white mustard the activity of POX decreased. The highest stimulation of peroxidase activity was found in pea (520% of POX activity in control plants) while in oat and agrimony the increase in CAT was 120%. This research has shown that fluoride probably causes generation of ROS and can either inhibit or stimulate antioxidant enzyme activity. This research was funded by the National Science Center of Poland, No. N N305 385138.

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## FLUORIDE-INDUCED CELL ULTRASTRUCTURE CHANGES

Life in plants and animals, including humans, depends on the orderly occurrence of a myriad of biochemical reactions which allow the harnessing of energy from the sun or food, the functioning of various organ systems, and the synthesis of an array of products including lipids, carbohydrates, and proteins. These reactions are sensitive to disruption by poisons such as cyanide ions which can, depending on the dose and route of administration, kill or cause chronic toxicity by inactivating cytochrome oxidase at cytochrome a<sub>3</sub>, thus uncoupling mitochondrial oxidative phosphorylation and inhibiting cellular respiration, even in the presence of adequate oxygen stores. The fluoride ion shares with the cyanide ion the capacity to impair the function of the mitochondrial enzyme cytochrome oxidase and to cause both acute and chronic toxicity. In addition, fluoride can, along with other actions, decrease the activities of mitochondrial succinate dehydrogenase and Mg<sup>2+</sup>-adenosine triphosphatase. These biochemical and physiological injuries become visible in symptoms of damage to plants or clinical illness in animals from fluoride toxicity. Their presence may be assessed by the measurement of various parameters such as enzyme levels, gene expression levels, and metabolite levels, and also by examining structural changes at the ultrastructural level. Chronic fluoride toxicity may cause a variety of nonspecific symptoms affecting a number of organ systems such as chronic fatigue not relieved by extra sleep or rest, headaches, dryness of the throat and excessive water consumption, urinary frequency, urinary tract irritation, muscle and joint aches and stiffness, muscle weakness and spasms, paraesthesiae, gastrointestinal disturbances, skin maculae and rashes, cognitive impairment, mood disturbance, balance difficulties, visual disturbances, brittle nails, repeated miscarriages or still births, male infertility, and tooth discolouration. Critics of the existence of the syndrome of chronic fluoride toxicity have considered that the so-called “fluoride intolerance” is in fact a

variety of unrelated conditions or has a psychogenic or psychosomatic basis. However, if an underlying cellular dysfunction, such as mitochondrial impairment, common to all the organ systems, was involved, a disturbance in many organ systems would be expected. The demonstration of disturbances at the cellular level, as detected by the measurement of various parameters and by observing ultrastructural changes, is a way of countering the argument that chronic fluoride toxicity is not an important issue for populations exposed to compulsory water fluoridation as occurs, in part or in all, of a number of countries including the USA, Canada, Iceland, Ireland, Israel, Australia, Singapore, and New Zealand. Ultrastructure studies have also been recommended in order to better understand the mechanisms of fluoride toxicity. In 1993, Chinoy, Sharma, and Michael stated that detailed studies, in fluoride toxicity, of the ultrastructure of muscle, especially its mitochondria were called for. Again in 1999, Chinoy and Patel noted that severe structural alterations had been found, with fluoride toxicity, in the mitochondria of guinea pig liver and in mice liver and kidney, and they called for further ultrastructural studies in the future. In reviewing the area, I found that ultrastructural studies have been made of the horse-bean plant, *Vicia Faba L.*, and various animal species including humans, mice, rats, rabbits, guinea pigs, pigs, and chickens. The animal tissues studied included the liver, pancreas, testis, submandibular gland, kidney, thyroid, myocardium, skeletal muscle, sciatic nerve, spinal cord, cerebellum, hippocampus, neocortex, femur, spleen, ovary, and uterus. In the plant study fluoride led to a high activity of the Golgi apparatus and an obvious destruction of the photosynthetic apparatus with the chloroplasts of the mesophyll cells showing lack of grana and dilation of the thylacoids. In the animal studies there were variable effects on the Golgi apparatus but typically the mitochondria were enlarged with disrupted cristae, and the rough endoplasmic reticulum was reduced in extent, irregular, dilated, and disrupted. For example, in a study of the cerebral cortex of human fetuses affected by fluoride toxicity, the nerve cells contained fewer mitochondria, granular endoplasmic reticulum, and ribosomes than the controls. The mitochondria showed marked swelling, the granular endoplasmic reticulum had expanded, and the nuclear membranes were damaged, with the contents of the nucleus spilling out of the nuclear envelope. Within the nucleus, there was an increase in heterochromatin, with some grouping around the edges. Synapses were relatively rare, and those noted were enlarged, with the synaptic membrane broken and fewer mitochondria, microtubules, and vesicles than usual. Some studies considered the time required for ultrastructural changes to occur. In the plant study destruction of the photosynthetic apparatus was apparent 24 hr after fumigation with hydrogen fluoride was initiated. In a human study of skeletal muscle two hours incubation with sodium fluoride was sufficient time to produce disintegration of the mitochondrial cristae and a decrease in the density of the mitochondrial matrix. In conclusion, ultrastructural studies of fluoride toxicity help elucidate the mechanisms by which the signs and symptoms of the condition emerge and the basis for many organ systems being involved.

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Keywords: Chronic fluoride toxicity; Endoplasmic reticulum; Mitochondria; Ultrastructural changes.

### **BENEFICIAL EFFECTS OF LIPOIC ACID AND SOME VITAMINS ON FLUORIDE TOXICITY IN LIVER OF RATS**

Experiments conducted in recent decades have provided valuable information about the influence of fluorides on the cellular respiration and on free radical processes that arise in excess from the harmful action of fluorides in cells. The aim of this study was to evaluate the effect of a diet supplemented with the antioxidative vitamins A and E and the nonenzymatic antioxidant lipoic acid on biochemical parameters and morphology of the liver after fluorine intoxication. The study was performed on 24 adult female Wistar rats divided into 4 groups. Rats from the control group were given distilled water. Rats from the first of the other three groups were given 10.0 mg NaF/kg bw/24 hr. Rats in the second of these three groups were given 70 mg lipoic acid/kg bw/24 hr. Rats in the third group received 3 mg vitamin E/rat/day, 250 IU vitamin A/rat/day and lipoic acid as the second group. The experiment lasted for 7 weeks. The rats were then intraperitoneally anaesthetized, using thiopental at a dose of 30 mg/rat. Upon dissection the livers were taken for histopathological examination and some fragments of organs for biochemical analyses. 10% of the parts served as homogenates in which the activity of protein metabolism enzymes such as: aspartate aminotransferase (AST), alanine aminotransferase (ALT),  $\gamma$ -glutamylotranspeptidase (GGTP) in rat liver were determined. Protein concentration and total antioxidative status (TAS) in liver were determined as well. The pathomorphological changes in the organs were assessed with preparations obtained with the normal paraffin method stained with hematoxylin and eosin (H-E). The results of examination indicated that fluoride ions interfere with protein metabolism. The slight changes in the activity of protein metabolism enzymes (decreased activity of AST, ALT, and GGTP) were accompanied by a statistically significant decrease of TAS concentration. Dispersed necrosis or focuses of necrosis comprising larger fragments of lobule were observed in a microscopic image of the liver. Besides, infiltrations from mononuclear cells, erythrorrhagia and hyperemia were also evident. The results show the positive, but not fully effective, influence of lipoic acid on the changes caused by fluoride in the rat liver. Total efficacy in preventing changes was achieved, however, by combined application of vitamins A and E and also by lipoic acid with them. These findings thus demonstrate the possibility of preventing adverse effects of exposure to fluoride through antioxidant dietary supplements.

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Keywords: Antioxidants; Fluoride toxicity in rats; Lipoic acid; Protein metabolism enzymes; Rat liver and fluoride; Vitamin A; Vitamin E.

### **ANALYSIS ON THE MONITORING RESULTS OF DRINKING WATER BORNE ENDEMIC FLUOROSIS IN CHINA (2009–2011)**

Since 2009, the Ministry of Health of the People's Republic of China has organized 29 provinces to carry out the monitoring of endemic drinking water borne fluorosis. The Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, organized this work, which was funded by the Chinese Ministry of Finance through the project of transfer payments. The purpose is to keep track of the progress of the implementation of water quality prevention measures and variations in the tendency for water-borne fluorosis in China. The progress of defluoridation and water supply projects was monitored in endemic fluorosis areas. Each province established a database using the village as a unit in all areas of endemic fluorosis. The information about each village was acquired by the investigation of Ministry of Water Resources. The operating condition of water supply projects and the fluoride content in drinking water were monitored. In the entire country, 136 counties were defined as monitoring regions according to stratified random sampling method. Ten projects were randomly selected from defluoridation and water supply projects in each monitoring county. For each project, the size and the operating condition were evaluated. The fluoride content of tap water was determined and the criteria were based on the sanitary standard for drinking water (GB5749-2006). Three villages were randomly selected as fixed monitoring stations in each monitoring county of fluorosis. Investigation of water supply projects and of the prevalence of dental fluorosis in each fixed monitoring station was conducted. Dental fluorosis was diagnosed by Dean's fluorosis index. In the first year, the survey for X-ray diagnosis of adult skeletal fluorosis was also carried out, and the diagnosis was based on the diagnostic criteria for endemic skeletal fluorosis (WS192-2008). By the end of 2010, there were 86,549 fluorosis villages in 28 provinces, involving 76.39 million people. Water supply projects have been completed in 70,832 fluorosis villages. The provision rate of water was 81.9%. Water supply projects were conducted in 32,524 villages of moderate and severe diseased areas, and the provision rate of water was 88.3%. In 2009, 1,393 water supply projects were monitored, in which 1,303 projects were under normal operation, accounting for 93.54%. Fluoride content in water of 959 projects was qualified with a pass rate of 68.84%. In 2010, 1,381 water supplies projects were monitored, in which 1,287 projects were under normal operation, accounting for 93.19%. Fluoride content in water of 1,504 projects qualified with a pass rate of 76.32%. In 2011, 1,408 water supplies projects were monitored, in which 1,334 projects were under normal operation,

accounting for 94.74%. Fluoride content in the water of 1,116 projects qualified with a pass rate of 79.26%. From 2009 to 2011, the detectable rates of children's dental fluorosis (8–12 years old) were 32.43, 26.97, and 24.98%, respectively, in the monitoring villages. In those villages, water supply projects were under normal operation and fluoride content in water was qualified. But in the villages without water supply projects, the detectable rates of children's dental fluorosis were 38.43, 46.08, and 45.51% respectively from 2009 to 2011. In 2009, 11,066 people in the villages with water supply projects were examined by X-ray, and the detectable rate of skeletal fluorosis was 16.28%. In the same year, 6,605 people in the villages without water supply projects were also examined by X-ray, and the detectable rate of skeletal fluorosis among them was 18.91%. Incidentally, the next monitoring of skeletal fluorosis will be conducted in 2014. In drinking water borne fluorosis areas of China, the provision rate of water was 81.9%. Overall, the rate of the water supply projects under normal operation has been maintained between 93 to 95%. The pass rate for the fluoride content in qualified drinking water has increased year by year. The pass rate increased from 68.84% to 79.26% in 2009 and 2011, respectively. After the provision of water, the detectable rate of children's dental fluorosis decreased significantly, 40% before the provision of water and 25% after the provision of water. Therefore, the implementation of safe drinking water projects in rural areas is effectively alleviating the hazards of drinking water borne fluorosis.

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Keywords: China; Drinking water-borne fluorosis; Monitoring; Water supply projects.

### **FLUORIDE CONTENT AND ANTIOXIDANT ACTIVITY OF INFUSIONS OF SELECTED HERBS FROM THE LAMIACEAE FAMILY**

Fluoride has important effects on human health, but the etiology of any disease related to its deficit in the body is still not known. Exposure to fluoride can cause fluorosis, inhibition of many enzyme activities, decrease in protein synthesis, and altered metabolism of carbohydrates and lipids. Fluoride can also have a significant effect on free radical processes and antioxidant enzyme activities. In recent years, interest in natural antioxidants has increased. This is important for two reasons. First, the daily human diet should be rich in components that protect the body from oxidative stress. Second, natural antioxidants can be added to food as an alternative to synthetic ones. Herbs, next to vegetables and fruits, have many biologically active substances, including antioxidants. Many species from the *Lamiaceae* family contain compounds, which have antioxidant properties. The aim of this study was to compare fluoride content and antioxidant activity of infusions obtained from herbs of *Lamiaceae* species. Fifteen samples of herb species were used: *Mentha piperita* (herba), *Lavandula angustifolia* (flos), *Origanum vulgare* (folium), *Satureja hortensis* (herba), *Satureja montana* (herba), *Ocimum basilicum* (herba), *Ocimum basilicum purpurea* (herba), *Melissa officinalis* (folium), *Salvia officinalis* (folium), *Leonurus cardiaca* (herba), *Thymus*

*vulgaris* (herba), *Hyssopus officinalis* (herba), *Rosmarinus officinalis* (herba), *Thymus citrodorus* (herba), and *Nepeta fasseni* (herba). All plant material was obtained from the Horticultural Experimental Station in Dołuje, Poland. A measured volume of boiled deionised water was added to 1 g of herbs and brewed for 5 or 15 min. Then infusions were filtered and cooled. The resulting extracts were used to analyse the fluoride content using the potentiometric method with an Orion ion-selective electrode and the percent of antioxidant activity by diphenylpicrylhydrazyl (DPPH) using the spectrophotometric method with a UV-1800 Shimadzu spectrophotometer. The experiment showed that 15-min infusions were characterized by higher fluoride concentration than those brewed only 5 min. The highest fluoride content of 15-min infusions was in *Salvia officinalis* (0.053 mg/L). However, in 5-min infusions the highest content of fluoride was observed of *Lavandula angustifolia* (0.048 mg/L). There were no significant differences in antioxidant activity between 5-min and 15-min infusions. The largest percent inhibition of the DPPH was observed in infusions of *Origanum vulgare*, *Satureja hortensis*, *Thymus citrodorus* and *Nepeta fasseni*. Moreover, in 15-min infusions a significant negative correlation between fluoride content and antioxidant activity was noted.

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Keywords: Antioxidant activity; DPPH (diphenylpicrylhydrazyl); Fluoride in herbs; Lamiaceae.

### **EXCESSIVE FLUORIDE EXPOSURE DELINEATING CHANGES IN DIFFERENT VITAMIN LEVELS AND OXIDATIVE BURDEN IN SCHOOL CHILDREN IN THE EASTERN REGION OF RAJASTHAN, INDIA**

Fluoride toxicity due to the presence of elevated levels of fluoride in drinking water (>1.5 ppm) may be a serious health problem of children and people in general. In the state of Rajasthan, almost all districts have high fluoride (up to 18.0 ppm) in their drinking/ground water sources, and about 11 millions of the populations are at risk. The aim of the present study was to investigate the status of vitamins A, E, C, and D, and minerals Cu, Zn, Fe, and Se in fluoride-exposed children 10 to 15 years of age. Thirty-two children were selected from a high-fluoride region of the eastern regions of Jaipur, where the mean fluoride content in water is  $5.5 \pm 1.2$  ppm. Age-matched controls were selected from the district where the fluoride content in water is less than 1.5 ppm. Three mL of blood sample were taken for estimation of vitamins and oxidative stress parameters such as lipid peroxide level (LPO), reduced glutathione (GSH) content, superoxide dismutase (SOD), and catalase activity. Determination of Cu, Zn, Fe, and Se in the plasma was conducted using atomic absorption spectrophotometry. A significant reduction in vitamin E, C, and D when compared with the age-matched controls was observed. On the other hand, LPO levels and Fe were higher. Depleted GSH,



Cu, Zn, and Se levels and lower SOD and catalase were observed in the fluorosis area subjects than in the controls. These results indicate that fluoride exposure promotes oxidative stress and alteration in vitamin and mineral levels. These changes may induce pathophysiological conditions owing to lack of vitamins and minerals. However, further in-depth studies are required for the understanding of the pathophysiology of fluorosis.

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Keywords: Children; Fluoride in water; India; Minerals in blood; Rajasthan, India; Vitamins in blood.

### **INCREASED OXIDATIVE BURDEN IN HYPERTENSIVE RETINOPATHY PATIENTS AND ITS ASSOCIATION WITH FLUORIDE EXPOSURE IN A POPULATION OF RAJASTHAN INDIA**

A high level of fluoride ingestion has a definite relationship to the prevalence and severity of diseases. There is limited study on the association of hypertensive retinopathy and fluoride exposure. We have recruited 55 patients with hypertensive retinopathy from the high fluoride region (>4.5 ppm) of Rajasthan along with 49 age- and sex-matched positive controls from a region where fluoride content in water was less than 1.5 ppm. Venous blood was collected for analysis of total reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl content (PC), and antioxidant activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In addition, total fluoride levels in blood and serum lipid profiles, viz., total cholesterol (TC), triglycerides (TG), and HDL- and LDL-cholesterol in hypertensive patients were examined and compared with those of the positive control. The results revealed that serum total cholesterol, triglycerides, and LDL-cholesterol were significantly elevated ( $p < 0.001$ ), whereas the level of HDL-cholesterol was significantly lower ( $p < 0.001$ ) in the hypertensive patients as compared to the controls. Reduced antioxidant enzyme activity and elevated levels of MDA and PC were also observed. Hypertensive retinopathy is therefore associated with higher blood fluoride content and with increased oxidative stress.

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Keywords: Fluoride in water; Hypertensive retinopathy; Oxidative stress; Rajasthan, India.

### **EFFECTS OF FLUORIDE ON NEUROTRANSMITTERS IN BRAIN REGIONS OF RATS EXPOSED TO FLUORIDE IN DRINKING WATER FOR TWO MONTHS**

Fluoride has long been known as an environmental pollutant. Toxic neurological effects of fluoride have been reported in experimental animals by oral exposure to fluoride, but effects on neural transmitters are not clear. Adult male rats were administered fluoride via their drinking water at concentrations of 0, 50, 100, and 200 ppm (as fluoride ion) for 2 months. Following the treatment period, the body weights of the rats were checked, and their brains, livers, kidneys, and spleens were sampled. The brain samples were dissected into seven regions: cerebrum, cerebellum, medulla oblongata, midbrain, corpus striatum, hypothalamus, and hippocampus. The levels of the catecholamines, norepinephrine (NE), dopamine (DA), DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovallic acid, and those of serotonin (5-hydroxytryptamine) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in each brain region, were simultaneously determined by high performance liquid chromatography (HPLC) with an electrochemical detector. The mean body weight in the 200-ppm group was significantly lower than in the control, 50-ppm, and 100-ppm groups. There were no significant differences among the groups for liver, kidney, or spleen weights. The mean values of NE in the medulla of the 100-ppm and 200-ppm groups were significantly lower than those in the control and 50-ppm groups. The mean values of 5-HIAA in the 100-ppm and 200-ppm groups were significantly lower than that in the 50-ppm group. However, they were also significantly lower than that in the control. The mean values of DA in the medulla in the 100-ppm and 200-ppm groups were significantly lower than that in the 50-ppm group and tended to be lower than that in the control. No significant differences were observed in the levels of the neurotransmitters and their metabolites in other brain regions. The mean value of DOPAC/DA ratio in the midbrain in the 50-ppm group was significantly higher than that in the control, 100-ppm and 200-ppm groups. The administration of fluoride at the concentration of 200 ppm in the drinking water for 2 months clearly inhibited increase in body weight. However, under the experimental conditions reported here, significant alterations in the neurotransmitters and their metabolites were observed in limited regions but were not dose dependent. The neurotoxic effects of fluoride by oral exposure in adult rats over a two-month period may therefore not be strong.

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Keywords: Neurotoxicity; Neurotransmitters; Subacute exposure.

### FLUORIDE EXPOSURE AND RISK OF SKELETAL FLUOROSIS AMONG AN ADULT POPULATION LIVING IN AN ENDEMIC FLUORIDE AREA OF ETHIOPIA

Adverse health effects of fluoride, particularly dental and skeletal fluorosis, caused by chronic exposure to excessive fluoride, continue to be major health problems in many parts of the world. Ethiopia is one of the severely fluoride-affected countries in Africa, where about fourteen million people are estimated to be at risk of fluorosis. The main objective of the present study was to characterize and determine fluoride exposure and total daily fluoride intake of the general population living in eight camps of the Metahara sugar estate area as well as risk of fluoride-related health effects among the adult population. The fluoride content of various drinking water sources (i.e., piped, well, and river water), beverages (i.e., tea and coffee), and the staple foods regularly consumed by the communities was determined. Fluoride was also analyzed in repeated urine samples collected from 430 adult participants. The total daily fluoride intakes and the risk of skeletal fluorosis among the population were estimated following health risk assessment methods. The mean drinking water fluoride concentrations in well, piped, and river water were measured as  $3.7 \pm 1.1$ ,  $0.84 \pm 0.12$ , and  $1.0 \pm 0.23$  ppm, respectively. Among the eight camps, Abadir II and Abadir IV were found to have the highest well water fluoride concentrations (mean  $4.2 \pm 0.8$  and  $4.9 \pm 0.68$  ppm, respectively). The staple foods such as, *Enjera* (made from teff, *Eragrostis tef*), bread (made from wheat), and *bulenta* (made from maize), were found to contain mean fluoride concentrations of 3.76 (range 0.88 to 8.4), 1.9 (range 1.2 to 4.1), and 2.8 ppm (range 0.9 to 5.8 ppm), respectively. The mean fluoride concentrations of different sauces consumed with enjera were measured as 4.2 (range 1.2 to 9.9) in a sauce made from beans (*shero*), 4.0 (range 1.9 to 7.9) in a sauce made from lentils, 1.8 (range 0.9 to 2.7), and 1.97 ppm (range 1.1 to 3.3 ppm) in mixed vegetables. Beverages, i.e., tea and coffee prepared and consumed by the populations were found to contain a mean fluoride concentration of 5.2 (range 2.7 to 12.5) and 2.4 ppm (range 1.2 to 6.1 ppm), respectively. The overall mean urinary fluoride concentration was 5.5 ppm (range 0.4 to 19.2 ppm). The highest mean urinary fluoride concentrations (mean:  $7.86 \pm 2.9$ ,  $7.49 \pm 3.28$ ,  $7.19 \pm 2.6$ , and  $6.6 \pm 3.1$  ppm)

were measured among participants living in Abadir III, Abadir II, Awash, and Abadir IV camps, respectively. Water fluoride concentrations were significantly correlated to urinary fluoride concentrations on a group basis when the mean water and mean urinary fluoride concentrations are compared ( $R^2 = 0.42$ ,  $p < 0.05$ ). All well water samples have fluoride concentrations above the WHO maximum level for fluoride of 1.5 mg/L (WHO, 2006). The urine fluoride levels were relatively high, and more than 80 percent of the participants exceeded a urine fluoride level (0.2 to 3.2 ppm), which is usually measured in non-occupationally exposed people of the general population. The staple foods, particularly *enjera* with *shero* and lentil sauces, and beverages, especially tea, contributed significantly to the total daily fluoride intake. A large part of the population living in the Metahara sugar estate area is therefore exposed to fluoride consumption through drinking water, food, and beverages at levels wherein there is a significant risk of fluorosis.

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Keywords: Ethiopia; Fluoride consumption; Fluorosis; Total daily fluoride intake.

#### **DOSE-RESPONSE RELATIONSHIPS BETWEEN DRINKING WATER FLUORIDE, BONE MINERAL DENSITY, AND SERUM OSTEOCALCIN**

Relationships between drinking water fluoride and bone mineral density (BMD), and serum osteocalcin (bone gla-protein, BGP) were examined to explore whether BMD and serum BGP are significant early screening biomarkers for fluorosis, especially for early bone damage in areas of endemic fluorosis. For this study, 103 adults were selected in 2006 from Wamiao village (severe endemic fluorosis area, as the fluoride exposed group) and 43 from Xinhuai village (nonendemic fluorosis area, as the control group) in Jiangsu Province, China, according to the drinking water fluoride in their household shallow well. The sex, age, body height, body weight, drinking water fluoride in each person's household well, BMD, and serum BGP were recorded, and the dose-response relationships between these parameters were analyzed using CurveExpert 1.3 Software. The fluoride levels of the drinking water of males and females in families in Wamiao ( $2.38 \pm 0.68$ ,  $2.62 \pm 0.91$  mg/L) were significantly higher ( $t = 14.27$  and  $11.08$ ,  $p < 0.01$ , respectively) than those in Xinhuai ( $0.35 \pm 0.08$ ,  $0.36 \pm 0.07$  mg/L). BMD in males in Wamiao ( $0.78 \pm 0.07$  g/cm<sup>2</sup>) was significant lower ( $t = 2.37$ ,  $p < 0.05$ ) than in Xinhuai ( $0.83 \pm 0.08$  g/cm<sup>2</sup>). Serum BGP in males and females in Wamiao ( $4.17 \pm 0.67$ ,  $4.11 \pm 0.57$  µg/L) were significantly higher ( $t = 17.64$  and  $19.40$ ,  $p < 0.01$ , respectively) than in Xinhuai ( $1.48 \pm 0.40$ ,  $1.44 \pm 0.39$  µg/L). BMD ( $0.66 \pm 0.15$  g/cm<sup>2</sup>) in the group with drinking water fluoride  $\geq 2.92$  mg/L was significantly lower ( $p < 0.01$ ) than in the group with drinking water fluoride  $< 0.42$  mg/L ( $0.76 \pm 0.12$  g/cm<sup>2</sup>). The levels of serum BGP in the groups with the drinking water  $\geq 0.42$  mg F/L,  $\geq 2.05$  mg F/L, and  $\geq 2.92$  mg F/L ( $3.83 \pm 1.07$ ,  $4.22 \pm 0.72$ , and  $3.99 \pm 0.63$  µg/L respectively) were significantly higher ( $p < 0.01$ ) than in the group with the drinking water  $< 0.42$  mg/L ( $1.44 \pm 0.37$  µg/L). The equation for the dose-response relationship between the drinking water fluoride and the rate of abnormal

BMD was  $y = (0.284 - 0.058x)^{-1.260}$ ,  $r = 0.99994$ ; and for the drinking water fluoride and the rate of abnormal serum BGP,  $y = 100.05 / (1 + 78.62e^{-4.55x})$ ,  $r = 0.99999$ . These significant dose-response relationships between drinking water fluoride and BMD and serum BGP indicated that BMD and BGP might be useful early screening biomarkers for endemic skeletal fluorosis, especially for bone damage.

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Keywords: Bone mineral density; Dose-response relationship; Osteocalcin; Water fluoride.

### FLUORIDE REDUCTION OF CONTRACTILE HEART FUNCTION IN RESPONSE TO ISCHEMIC STRESS

Accumulating studies show that excessive F exposure causes a variety of pathological changes in various tissues including the heart. In this study, we investigated the effect of sodium fluoride (NaF) on cardiac function in a murine heart with a pressure-volume conductance system and an *ex vivo* heart perfusion system. Adult male C57BL/6 mice were administered NaF by gavage for 4 weeks at concentrations of 30 mg/mL, 70 mg/mL, and 150 mg/mL, corresponding to  $2.84 \pm 0.29$ ,  $6.28 \pm 0.61$ , and  $14.18 \pm 1.00$  mg F/g bw/day. The pressure-volume loop data showed that NaF treatment significantly modulated the heart rate (HR) and cardiac output (CO) of the mouse heart in a dose-dependent manner. The low NaF dosage (30 mg/mL) did not alter cardiac function, whereas the high NaF dosages (70 and 150 mg/mL) reduced HR and CO of the mouse heart ( $n = 4-6$  per group,  $p < 0.05$  vs. vehicle control). To further address whether NaF altered the left ventricular contractile function, isolated mouse hearts from these groups were subjected to 20-min global ischemia followed by 30-min reperfusion in an *ex vivo* perfusion system. Intriguingly, the results demonstrated that post-ischemic left ventricular function was improved by NaF treatment (150 mg/mL) when compared to vehicle control group ( $135 \pm 5\%$  vs.  $75 \pm 0.7\%$  of baseline,  $p < 0.05$  vs. control group). However, a medium dosage of NaF (70 mg/mL) significantly decreased post-ischemic left ventricular contractile function ( $23 \pm 0.4\%$  vs.  $75 \pm 0.7\%$  of baseline,  $p < 0.05$  vs. control group) with no changes in the low dosage group. Taken together, these findings suggest fluoride reduced contractile function of the heart in response to ischemic stress, and they warrant further investigation regarding the effects of fluoride on cardiovascular health.

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Keywords: Cardiac study; Contractile function; *Ex vivo* heart study; Fluoride and heart; Heart health; Ischemic stress; Mouse heart.

### EFFECT OF FLUORIDE ON APOPTOSIS AND EXPRESSION OF CASPASE-3 AND CASPASE-9 IN RAT OSTEOBLASTS

Long-term and excessive fluoride (F) intake can cause multiple skeletal diseases, disturbing the balance of bone modeling and remodeling activities that are regulated by the action of various bone cells. Osteoblasts (bone-forming cells) play a leading role in this balance. The role of F in cytotoxicity and induction of apoptosis of these cells was investigated by treating neonatal rat osteoblast cultures with varying concentrations of sodium fluoride (NaF), from 0 mg/L to 30 mg/L for 24 and 72 hr. Proliferation of osteoblasts was measured by methyl thiazolyl tetrazolium (MTT) assay. At the end of 24 and 72 hr, the levels of apoptosis were analyzed with a fluorescence-activated cell sorter (FACS) by the Annexin-V-FITC and the propidium iodine (PI) double staining method. The expressions of caspase-3 and caspase-9 were determined by quantitative real-time polymerase chain reaction (QRT-PCR). The results indicated that NaF inhibited cell proliferation at higher concentrations and enhanced the amount of apoptosis with increasing dosages of NaF. At a lower concentration of NaF (5 mg/L), the expressions of the caspase-3 and caspase-9 increased after 72 hr. The results suggested that F up-regulates the expression of caspase-9, which subsequently activates caspase-3, thereby causing apoptosis in osteoblasts after 72 hr.

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Keywords: Apoptosis of osteoblasts; Caspase-3; Caspase-9; Fluoride and bone; Rat osteoblasts.

### SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN HERBAL PLANT DISTILLATES

Herbal distillates are used in abundance as beverages in Iran. For the first time the amount of fluoride in these distillates was determined. Sixty samples of twenty of the most commonly used herbal distillates (fennel, fenugreek, ginger, camel's thorn, cardamom, rice bran, citrus, licorice, aloe vera, eglantine, coriander, lemon balm, hawthorn, pussy, cinnamon, thyme, rosewater, alfalfa, olive leaf, and malva) were selected for analysis. The fluoride content of each sample was measured three times with SPADNS by spectrophotometry (model Dr4000, HACH Co.). The lowest concentrations of fluoride were found in ginger, eglantine, hawthorn, thyme, licorice, olive leaf (mean = 0 mg/L) and the highest in alfalfa (mean = 0.32 mg/L) samples. The results indicated that fluoride levels in the herbal distillate samples are low and therefore pose no risk of fluorosis.

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Keywords: Fluoride in beverages; Iran; Herbal plant distillates; Spectrophotometric analysis.

## **DEFLUORIDATION OF GROUND WATER BY POLYALUMINUM CHLORIDE (PAC)**

This study investigated the reduction of groundwater fluoride by the chemical coagulant polyaluminum chloride (PAC, 99%) in the drinking water network system of Sari City of Mazandaran Province located in northern Iran. The efficiency of PAC as a coagulant to remove fluoride from water was investigated for the effects of PAC dosage, different pH, mixing speed, and initial amount of fluoride. The Jar test with synthetic solutions of fluoride in distilled water was used at 80–100 rpm for initial rapid mixing and then set at 30 rpm. Samples were withdrawn at 20, 40, 60, 80, and 120 min. The resulting level of fluoride was measured by the SPADNS method with a DR 2000 spectrophotometer. The best dosage of PAC was ~1 g/L at pH 7 for 24.14 mg/L of initial fluoride concentration. An efficiency of 51% was achieved under these conditions. This study has shown that PAC for removal of fluoride at high concentration is appropriate. Furthermore, it is less expensive than other absorbents.

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Keywords: Defluoridation; Groundwater fluoride; Polyaluminum chloride; Sari City, Iran.

## **FLUORIDE REMOVAL FROM WATER BY ALUM COAGULANT**

Fluoride in high concentration can cause adverse health impacts, especially for babies. According to the WHO guideline, fluoride concentration in drinking water should be limited to 1.5 mg/L. In this work, the efficiency of alum as a coagulant to remove fluoride from water was investigated. The effects of chemical dosage of alum at different pH, mixing speed, and initial amount of fluoride were studied. Experiments were conducted in three stages, i.e., selecting the best dosage, best pH, and the best initial fluoride concentration. Results showed that the best alum dose was 250mg/L at pH 2.6, and the maximum efficiency was 97% for an initial fluoride concentration of 14.4 mg/L. Fluoride removal efficiency decreased with increasing levels of initial fluoride, yet at low concentrations of fluoride alum is an efficient and inexpensive compound for removal of fluoride from water.

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Keywords: Alum coagulant; Fluoride removal; Water treatment.

## **DETERMINATION OF THE 50% PHYTOTOXICITY THRESHOLD FOR DIFFERENT FLUORIDE CONCENTRATIONS AFFECTING THE SPRING WHEAT (*TRITICUM AESTIVUM* L.) AND WHITE MUSTARD (*SINAPIS ALBA* L.) SEEDLINGS**

Modern environmental pollution by fluoride is a major problem because of its toxicity to plants, animals, and humans. Some plants are extremely sensitive to fluoride accumulation and display important signs of changes in the environment. Therefore it is important to continuously monitor the level of fluorine compounds, especially in sensitive plant species in ecosystems for changes in biochemical, physiological, histological, and genetic parameters. The aim of this study was to

determine the concentrations of fluoride in the soil that cause 50% root growth inhibition of two plant species: white mustard (*Sinapis alba* L.) and spring wheat (*Triticum aestivum* L.). The study was conducted in 2011 in the laboratory of the Department of Biochemistry, West University of Technology in Szczecin. Soil samples characterized by granulometric composition of light clay and 1.10% organic carbon were collected from the black-earth of Gumieniecka Plain. The phytotoxicity test consisted of two phases. Preliminary studies were performed to determine the concentration range of fluoride compounds that affect the quality of the soil. Therefore, according to the ISO-11269-2:1995 method, NaF was introduced into the soil at concentrations of 0, 1, 10, 100, and 1000 mg F<sup>-</sup>/kg dm (dry matter) soil. Further studies were conducted with additional fluoride concentrations of 200, 400, 600, and 800 mg F<sup>-</sup>/kg dm soil. Two plant species were used: a monocotyledonous plant—spring wheat (*Triticum aestivum* L.) and a dicotyledonous plant—white mustard (*Sinapis alba* L.). From our study we found that 50% inhibition of root growth was caused by 441 mg F<sup>-</sup>/kg dm soil in case of white mustard seedlings and by 503 mg F<sup>-</sup>/kg dm soil in spring wheat seedlings. However, we also found that as little as 1 mg F<sup>-</sup>/kg dm soil stimulated root growth of spring wheat.

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Keywords: Fluoride phytotoxicity; Mustard plant, *Sinapis alba*; Spring wheat, *Triticum aestivum*.

### **INCREASE OF FLUORIDE TOXICITY BY SULFUR DIOXIDE IN MALE RODENT REPRODUCTION ASSOCIATED WITH MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNAL PATHWAYS**

Fluoride and sulfur dioxide are two-well known environmental pollutants, sometimes co-existing, which can present a serious threat to public health. In order to explore the potential toxic effects of these two pollutants on male reproduction and the mechanism of their interaction, sexually mature rats and unmatured mice were treated with fluoride in their drinking water (100 mg NaF/L) and sulfur dioxide in their air (39.3 mg SO<sub>2</sub>/m<sup>3</sup> for 4 hr/day), and also with 150 mg NaF/L in their drinking water and 26.2 mg SO<sub>2</sub>/m<sup>3</sup> for 3 hr/day. Application of diverse methods as radioimmunoassay, transmission electron microscopy, flow cytometry (FCM), terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL), immunohistochemistry, and Real-time PCR provided data for assessing changes in sperm quality, morphology and subcellular structure of testis, protein level, antioxidation and the activity of relative enzymes in testis, apoptosis in seminiferous cell, and the expression of p53, bcl-2, bax gene in these two kinds of models. Moreover, miRNA microarray, a new type of technology, was used to examine the changes of non-coded RNA in order to study the mechanism of effects of fluoride and SO<sub>2</sub> on spermatogenesis. Afterwards, the mitogen-activated protein kinase (MAPK) pathway (ERK1/2, JNK1/2/3, p38 and ERK5) and transcriptional factors of p38 downstream (GADD153, MAX, MEF2C and Sap1a)



expression levels in mice testis were assessed according to the results of bioinformatics analysis. The results indicate that SO<sub>2</sub> enhances the damage caused by fluoride in the male reproductive system, including the changes in structure of tissues, cell and molecular level, to significantly impair reproductive function. The combined toxic effect may involve oxidative stress in testis tissue, disruption of the DNA single chain, activation of DNA-PK and induce phosphorylation, and activation of p53 protein. Furthermore, the activated p53 protein can enhance the expression of the apoptosis genes bcl-2, bax et al. and initiate apoptosis of spermatogenic cells, finally impairing spermiogenesis. Moreover, miRNAs and MAPK signal pathways may play the key role during events leading to the injury. Acknowledgements: This research was sponsored by the China National Natural Science Foundation (Grant No. 30671545; 30901092 ; 31172380 ; 31072180), the Specialized Research Fund for the Doctoral Program of Higher Education of China (Grant No. 20091403120002), Natural Science Foundation for Young Scientists of Shanxi Province (Grant No. 2009021035-1), Shanxi Province Provincial Foundation for Leaders of Disciplines in Science (Grant No.201001).

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Keywords: Male reproduction function; Immature male mice; MAPK signal pathways; Mature male rats; MicroRNA; Spermatogenic cells; Sulfur dioxide.

## **DENTIN HYPERSENSITIVITY IN AN ENDEMIC FLUOROSIS RURAL AREA OF CHINA**

Dentin (or dentine) hypersensitivity is a transient sharp, well-localised pain arising from exposed dentin, typically in response to chemical, thermal, tactile, or osmotic stimuli, which cannot be explained by any obvious dental defect or pathology. According to a recent survey, dentin hypersensitivity is a common oral problem among adults in China with a prevalence of about 29.7% in urban adults and 34.8% in rural adults. Fluoride is widely considered an effective agent to prevent dental caries, but excessive exposure to fluoride, especially from drinking water, can cause dental fluorosis and adversely alter enamel structure. Although there are many studies about dentin hypersensitivity with different survey methods and different target populations in the world, we are not aware of any on dentin hypersensitivity in an endemic fluorosis rural area. The aim of this study was to compare the prevalence and clinical features of dentin hypersensitivity in subjects with and without fluorosis. A total of 1048 adults, aged 30–69 years (622 in an endemic fluorosis rural area and 426 in a nonendemic fluorosis rural area) were examined at eight investigation centers in a one-year study in Liaoning Province of China. Participants completed questionnaires and underwent clinical examination. Dentin hypersensitivity was diagnosed by recording the reaction after stimulation with a blast of compressed air from a triple syringe held 1 cm

from the tooth surface. Relative risk factors of dentin hypersensitivity were analyzed. A fluoride ion selective electrode was used to determine fluoride content in drinking water, which ranged from 1.15 to 1.50 mg/L in the endemic fluorosis rural area and from 0.37 to 0.75 mg/L in the nonendemic rural area. The prevalence of dental fluorosis in the endemic fluorosis rural area was 95%, and 61 of 622 (9.9%) individuals in that area were found to have dentin hypersensitivity. The most-affected teeth were the lower front teeth. In the nonendemic area, 122 of 426 subjects (28.6%) were affected by dentin hypersensitivity, mostly in the premolar teeth. Thus, there was significant difference between the two areas. The questionnaires, however, showed no difference in the life style and oral hygiene habits between the two areas. No one in the sensitive group had taken medication or gone to see a dentist. The subjects in the endemic fluorosis rural area may have been suffering less from dentin hypersensitivity than the subjects in the nonendemic areas. Public oral health education and effective treatment of dentin hypersensitivity are suggested for Chinese rural areas.

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Keywords: China; Dentin hypersensitivity; Endemic fluorosis areas; Fluoride in water; Rural areas.



Photograph courtesy of B Spittle

Baltic seacoast, Poland. Poland is bordered on the northwest by the Baltic Sea along a 524 km coastline.

**INAUGURAL PRESENTATION AT THE XXXTH CONFERENCE OF THE  
INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH,  
ADVANCES IN FLUORIDE RESEARCH,  
SEPTEMBER 5–8, 2012  
SZCZECIN, POLAND**

**FLUORIDE TOXICITY FUELED BY MODERN LIFESTYLE**

According to results of modern anthropological studies, the origin or evolution of mankind (humankind, *Homo sapiens*) from primitive tool-making hominids on planet Earth extends back several million years. With the passage of time and the appearance of *Homo sapiens*, exposure to toxic materials in the environment has been a constant threat to survival. However, mankind has survived because the human body is able to compensate for negative impacts as long as sufficient positive factors are present to maintain health in the human body. This principle of nature has worked for eons of time—until the last century!

Today there is a growing body of alarming evidence that modern chronic diseases like myocardial infarction, stroke, diabetes, osteoporosis, and cancer, plus neurological disorders like attention deficit syndrome, multiple sclerosis, and dementia, are promoted and may be even due to modern technical achievements that have profoundly changed human lifestyle in two ways: First, there is a massive destruction of the environment necessary for our survival by an unprecedented growth in exposure to a large list of pesticides and toxic materials like fluoride and aluminum. Second, technological progress has led to a sedentary lifestyle and the extensive consumption of often-unhealthy processed foods.

As a consequence, these changes have led to a serious loss of essential ingredients for good health in large segments of the population: physical exercise, micronutrients, essential fatty acids from plants and animals, and vitamin D—the “sunshine” hormone—as well as the dissipation among many people of close social bonds and of performing music and singing, to mention but a few. The deficit of these resources reduces the health of our bodies, not only in the last decades of life but also in the early stages of life, by disruption of fetal programming leading to inheritable changes of our genes. This phenomenon is part of the new field of scientific studies in “epigenetics” research.

Hard to believe, but obviously true: one way to avoid or solve various health problems is a change of lifestyle including a simple nutritional approach! Our beloved late colleague, Professor Emerita Niloufer J Chinoy, Gujarat University, Ahmedabad, India (October 17, 1939 – May 8, 2006), gave an important example: eating the right combination of fresh foods with high antioxidant capacity significantly helps to compensate against many of the adverse effects of endemic fluorosis.

*Conclusion:* Chronic diseases are the dominant health problem in the 21st century worldwide. Scientific effort should not only focus on avoiding negative factors like pesticides, fluoride, and aluminum, but it should also press for greater attention to compensating factors in case of inevitable exposure. This can be done

by remembering and restoring the natural resources mankind has lost so extensively as a result of technological progress and lifestyle changes.

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Photograph courtesy of B Spittle

The Cloth Hall in Kraków's Main Market Square was built in the 14th century as a centre for the cloth trade but was burnt down in 1555 and then rebuilt in Renaissance style. The arcades were added in the late 19th century. The square, designed in 1257, measures 200 m by 200 m and is the largest medieval town square in Poland. Although the buildings in the square appear neoclassical their basic structures are much older with their cellars dating from medieval times.



Photograph courtesy of B Spittle

St Mary's Church in Kraków's Main Market Square is a brick Gothic church re-built in the 14th century to replace an earlier church built in the 1220s which was destroyed in Tatar raids. The church's altarpiece is a pentaptych, intricately carved in lime wood, painted, and gilded, with a central panel and two pairs of side wings that measures 13 m high and 11 m wide making it the largest piece of medieval art of its kind.