COMPARATIVE ANALYSES OF HEAVY METALS IN PORTLAND CEMENT, PROROOT MTA, ANGELUS MTA USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

Dissertation Submitted to

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In Partial Fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

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CERTIFICATE

This is to certify that this dissertation titled **"Comparative Analyses of Heavy Metals in Portland Cement, Proroot MTA, Angelus MTA using Atomic Absorption Spectrophotometry"** is a bonafide record of work done by **DR. R. JEGATHEESAN** under our guidance during the study period 2009-2012.

This dissertation is submitted to **THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, (BRANCH IV)**. It has not been submitted (partial or full) for the award of any other degree or diploma.

Guided By:

RAGAS DENTAL COLLEGE & HOSPITAL CHENNAI

Dr. R. Anil Kumar, MDS
Professor
Department of Conservative
Dentistry & Endodontics
Ragas Dental College &
Hospital
Chennai

Dr. R. Indira, MDS
Professor & Head of Dept.
Department of Conservative
Dentistry & Endodontics
Ragas Dental College &
Hospital
Chennai

Dr. R. INDIRA.

Dr. S. Ramachandran, M.D.S.,
Professor and Principal,
Department of Conservative Dentistry & Endodontics
Ragas Dental College and Hospital, Chennai

PRINCIPAL
RAGAS DENTAL COLLEGE & HOSPITAL
CHENNAI

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INTRODUCTION

An ideal orthograde or retrograde filling material should seal the pathways of communication between the root canal system and its surrounding tissues. It should also be nontoxic, noncarcinogenic, nongenotoxic, biocompatible with the host tissues, insoluble in tissue fluids, and dimensionally stable[31,12.] Because existing restorative materials used in endodontics did not possess these ‘‘ideal’’ characteristics[31], mineral trioxide aggregate (MTA) was developed and recommended initially as a root-end filling material and subsequently has been used for pulp capping, pulpotomy, apexogenesis, apical barrier formation in teeth with open apices, repair of root perforations, and as a root canal filling material[36]. MTA has been recognized as a bioactive material that is hard tissue conductive, hard tissue inductive, and biocompatible.

The material was first developed at Loma Linda University for use as a root end filling material by Torabinejad in 1993. MTA has been patented and has received the approval of the Federal Drug Administration (FDA) in the USA. The crystalline material was essentially calcium oxide and amorphous calcium phosphate.

Hydration of MTA powder resulted in the formation of colloidal gel that hardened. The pH of MTA immediately after mixing was 10.2, raising to 12.5 after 3 hrs[30]

The composition of MTA is Calcium oxide (from limestone) which makes up 50–75 mass%, while Silicon dioxide and Aluminium trioxide (both from clay or shale) constitute 15–25mass% and 2–5mass% respectively Magnesium oxide, Ferric oxide, and trace amounts of other heavy metals are also reported to be found.[17] It has been reported that Portland cement type 1 is the main component of MTA with addition of bismuth oxide at 4:1 ratio to provide radiopacity[30]. The addition of 20% bismuth oxide to Portland cement increases the radiopacity value to 6–8 mm Al. There are some differences among published studies regarding the chemical composition of MTA. These differences are related to the various liquids used to mix with MTA powder[17, 30] and various equipments used to test its composition.

Different brands of MTAs available in the market are Proroot MTA (Dentsply), MTA Angelus. Chemically two different type of MTA are available ie. Grey MTA (GMTA) and White MTA (WMTA). The main difference in chemical composition between GMTA and WMTA is a significant

decrease in the amount of iron and other metallic elements in the latter. MTA and Portland cement have almost identical properties macroscopically, microscopically, and by x-ray diffraction analysis.[17]

MTA is very similar to Portland cement, which is used to manufacture concrete for the construction industry. The commercial versions of MTA were shown to have broadly similar constitution to ordinary Portland cement except for the addition of bismuth oxide. Portland cement is manufactured by mixing calcareous materials and argillaceous materials in a kiln at 1,450°C producing a clinker, which is eventually ground with gypsum-forming cement. Portland cement has been suspected to contain undesirable contaminant substances, thus being undesirable for use in humans. The resultant cement may contain some heavy metal contamination in amounts ranging from 5 to 100 parts per million (ppm). These heavy metals include arsenic, chromium, and lead, which are incorporated in the cement during its production.[27]

The heavy metals are incorporated from the substitution of primary fuels to reduce costs with alternate fuels, which are usually wastes, and also by the utilization of wastes, an

alternative inorganic raw material together with the limestone and shale. [27] Waste used as an inorganic raw material or used as a secondary fuel may contain widely varying metal concentrations compared with raw materials and regular fuels. Trace elements found in wastes that are used in cement production may either be transferred to the cement or emitted with exhaust gases into the environment. The details of the manufacture of MTA were Scarce.

Systemic effects are those that require absorption and distribution of the toxicant to a site distant from its entry point, at which point effects are produced. Most chemicals that produce systemic toxicity do not cause a similar degree of toxicity in all organs, but usually demonstrate major toxicity to one or two organs.

The systemic toxicity of a material depends on factors such as physical state, gas, solution, or powder particle size, the rate of absorption into cells, the rate of elimination, the nature of chemical substituents in the toxic compound, and, of course, the pre-existing state of the patient.[22]

A Local effect refers to an adverse health effect that takes place at the point or area of contact. The site may be skin, mucous membranes, the respiratory tract, gastrointestinal system and eyes.

The **trivalent Arsenic** species of oxidation is the most toxic. In human adult subjects, the lethal dose range of inorganic arsenic is estimated to be 1–3 mg/kg.[20]**Hexavalent chromium** is recognized as an environmental and occupational carcinogen[22]. **Lead** has been used heavily in tile industry. CNS is the principal target organ for lead in the developing children. Lead readily enters the brain and is selectively deposited in the hippocampus and cortex.[21]

No specifications for arsenic, lead, chromium or other heavy metals are listed in cement certifications. ISO 9917-1 (2007)[5] specifies a maximum value of 2 mg/kg and 100 mg/kg for acid-soluble arsenic and lead, respectively, for restorative dental materials.

The inclusion of heavy metals in MTA is of concern because they come in contact with hard and soft tissues.

The aim of this present study was to investigate the levels of Heavy metal contents present in Portland cement, Proroot MTA, and Angelus MTA.

Objective of this present study was to

1. Quantify the levels of the acid soluble arsenic, lead, and chromium content of Portland cements, Proroot MTA and Angelus MTA

2. Investigate and compare the leaching of the metal ions of Arsenic, lead and Chromium in deionized water and Simulated Body Fluid using atomic absorption Spectrophotometry.

REVIEW OF LITERATURE

Kimberlie A(1998)[18] reviewed the heavy metal toxicity of lead and metal fume fever to identify the toxic pathophysiology, clinical presentation, and emergency department management of lead toxicity and metal fume fever. He summarized that however, early recognition of these poisonings is essential for efficacious treatment and epidemiologic tracking of accidental exposures. Signs and symptoms are nonspecific, making a thorough history and physical examination essential in achieving an accurate diagnosis. Heavy metal poisonings are optimally managed in consultation with a toxicologist or regional poison center.

Michael F. Hughes (2002)[20] investigated the Arsenic toxicity and potential mechanism of action. The metabolism involves reduction to a trivalent state and oxidative methylation to a pentavalent state. The trivalent Arsenicals, including those methylated, have more potent toxic properties than the pentavalent Arsenicals. The exact mechanism of the action of Arsenic is not known, but several hypotheses have been proposed. At a biochemical level, inorganic Arsenic in the pentavalent state may replace phosphate in several reactions. In the trivalent state, inorganic and organic (methylated) Arsenic may react with critical thiols in proteins and inhibit their activity. Regarding cancer, potential mechanisms include

genotoxicity, altered DNA methylation, oxidative stress, altered cell proliferation, co-carcinogenesis, and tumor promotion.

Norwood.W.P(2002)[23] investigated the Chronic toxicity of As, Co, Cr and Mn to Hyalella Azteca can be described using a saturation based mortality model relative to total-body or water metal concentration. He found that the LBC25s (total-body metal concentrations resulting in 25% mortality in 4 weeks) were 125, 103, 152 and 57,900 nmol g^{-1} dry weight for As, Co, Cr and Mn respectively. He observed the hormesis growth response to As exposure. He concluded that Bioaccumulation of As, Co, Cr, and Mn was strongly correlated with, and is useful for predicting, chronic mortality.

Antonio Hungaro Duarteet al (2005)[15] determine the release of Arsenic from 2 gray Portland cements, a white Portland cement, and 2 MTAs (ProRoot and MTA-Angelus). The materials were manipulated and placed in plastic tubes, and the tubes were immersed in glass flasks containing water with grade reagent, pH 5.0. After 3 and 168 h, the water in which the material had been immersed was analyzed regarding the presence of Arsenic by atomic absorption spectrophotometry with hydride generation. The results showed that the levels of Arsenic released were similar for Portland cements and MTAs, and were well below those considered to be harmful.

Camilleri et al (2005)[7] evaluated the biocompatibility of mineral trioxide aggregate and accelerated Portland cement and their eluants by assessing cell metabolic function and proliferation. The chemical constitutions of gray and white Portland cement, grey and white MTA and accelerated Portland cement produced by excluding gypsum from the manufacturing process (Aalborg white) was determined using both energy dispersive analysis with X-ray and X-ray diffraction analysis. Biocompatibility of the materials was assessed using a direct test method were cell proliferation was measured quantitatively using Alamar Blue TM dye and an indirect test method where cells were grown on material elution and cell proliferation was assessed using methyltetrazolium assay. Biocompatibility testing of the cement eluants showed the presence of no toxic leachables from the grey or white MTA, and that the addition of bismuth oxide to the accelerated Portland cement did not interfere with biocompatibility. The elution made up of calcium hydroxide produced during the hydration reaction was shown to induce cell proliferation.

Santos et al (2005) reported the results of the studies on Ca^{2+} release, pH and electrical conductivity of experimental cement and compared them with those of MTA angelus. Five samples of each cement were prepared using plastic tubes 1mm is diameter and 10mm long. Each sample was sealed in a test tube containing 10ml de ionized water which was analyzed after, 24, 48, 72, 96, 192, 240,

& 360 hrs for PH, electrical conductivity and calcium release. The concentration of calcium is obtained through atomic absorption spectroscopy. The experimental cement released calcium and hydroxyl ions comparable with those released by MTA angelus. After 24 hrs the calcium ion release by Electric conductivity was greater than MTA and two cements released the ions up to 360h storage in aqueous solution.

Astary et al (2005) conducted the study to analyze and compare the elemental constituents of WMTA and GMTA. Each cement was mixed with distilled water according to manufacturer's instruction and placed in the cavities of depth 3 mm with appropriate condenser. The samples were immersed in saline and allowed to set in incubator at 37 degree c for 48 hrs. The elemental composition was determined using SEM equipped with light element energy dispersive spectrometer. The results showed that WMTA has smoother mixture, GMTA shows bigger crystals. The study concluded the observed concentration for Al2O3, MgO and particularly FeO in white MTA are considerably lower than those found in GMTA. The observed concentration of FeO is GMTA thought to be the primary response for variation in colour.

G. De Deus et al in (2005)[14] evaluated the cytotoxic effects of two brands of MTA (pro-Root MTA and MTA Angelus) and portland cement (PC) on the human ECV 304 endothelial cell line.

Endothelial ECV 304 cells were incubated at 37 degree C in atmosphere of 95% air and, 5% carbon dioxide and 100% humidity for 7 days and grown in F12 medium supplemented with 10% fetal bovine serum with 50 micro gram per mL of gentamicin sulphate. Effects of the materials on mitochondrial functions were measured by a colorimetric assay. Results showed that no statistically significant difference was shown between any of the experimental materials (9p>0.0t5).

Saeed Asgary (2005)[1] proposed a research to determine and compare the composition of white mineral trioxide aggregate and gray mineral trioxide aggregate. Electron probe microanalysis results indicated that lime (CaO), silica (SiO_2), and bismuth oxide (Bi_2O_3) were the dominant compounds in each case and were present at comparable levels in either of the types of mineral trioxide aggregate analyzed. It was concluded that the most significant differences observed were between the measured concentrations of Al_2O_3 (122%), MgO (130%), and especially FeO (1000%) when gray mineral trioxide aggregate was compared with white mineral trioxide aggregate.

Brian Bozeman et al (2006)[3] compared the Crystal growth and elemental dissolution characteristics of gray Mineral Trioxide Aggregate (GMTA), white MTA (WMTA), and an experimental material, Dentalcrete. The study consisted of part A, comparing

amount and composition of surface crystal growth, twelve cylinders of each material were suspended in Phosphate Buffered Saline (PBS) solution without Ca. The crystals were analyzed by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). For part B, three cylinders of each material were suspended in distilled, deionized water. The water was analyzed by ICP-AES for Ca content at 24 h, 72 h, and 5, 7, 10, and 14 days. Data were analyzed using one-way ANOVA and Tukey test. The results showed that Both MTA materials released more Ca initially, followed by a decline and then rise in elution. GMTA produced the most surface crystal, which may be clinically significant. The crystals on GMTA and WMTA were chemically and structurally similar to hydroxyapatite (HA).

Islam et al, in (2006)[17] conducted study to use X-Ray diffraction to compare the major constituents present in PMTA, white MTA, ordinary Portland and white portland cements. Specimens were prepared by packing dry powder into an X-ray holder which was placed on a flat glass slab. X-ray diffractometry of the 4 materials were carried out. The main constituents were found to be tri calcium silicate and tetra calcium alumino ferrite in all four cements with the additional presence of BI_2O_3 in pro root MTA and pro root MTA tooth coloured.

J. NING and M. H. GRANT (2006)[22] investigated the chromium induced cytotoxicity to osteoblast derived cells. He has investigated the cytotoxicity of Cr VI in immortalized rat osteoblast cells in vitro using alkaline phosphatase (ALP) activity as an index of toxicity. Cr VI caused a concentration - dependent decrease in ALP activity, thought to be mediated by intracellular reduction to Cr III. He found that the depletion of intracellular GSH by buthionine sulfoximine increased the toxicity of Cr VI at early time points (after 1.5 and 3 hr exposure). GSH and Cr VI therefore interact in the osteoblasts, and this may be through formation of a conjugate and/or by detoxication of reactive intermediates formed during redox cycling of the chromium.

Marília Gerhardt de. et al (2007) has analyzed and compared Portland cement to mineral trioxide aggregate (MTA) because of their chemical similarity. In view of this, the present study compared the components of a Portland cement (Votoran®) to two commercial brands of MTA (Pro-Root™ and MTAAngelus ®). Twelve specimens of each material were fabricated and examined by scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) to obtain their percentage of chemical elements. The means of the chemical elements found in each material was compared by descriptive statistics. The results showed that Bismuth was present only in MTA cements to provide radiopacity. In conclusion, the tested cements have similar components, which

supports, as far as composition is concerned, the possible clinical use of Portland as an option to MTA.

J. Camilleri et al, (2007)[8] reported the hydration mechanism of white MTA. The Chemical constitution of white MTA was studied by viewing the powder in polished section under the scanning electron microscope (SEM), the hydration of both white MTA and white Portland cement (PC) was studied by characterizing cement hydrates viewed under the SEM, plotting atomic ratios, performing quantitative energy dispersive analyses with X-ray (EDAX) and by calculation of the amount of anhydrous clinker minerals using the Bogue calculation. Results showed that Un-hydrated MTA was composed of impure tri-calcium and di-calcium silicate and bismuth oxide. The aluminate phase was scare. On hydration the white PC produced a dense structure made up of calcium silicate hydrate, calcium hydroxide, monosulphate and ettringite as the main hydration products. The un-reacted cement grain was coated with a layer of hydrated cement. In contrast MTA produced a porous structure. Bismuth oxide was present as un-reacted powder but also incorporated with the calcium silicate hydrate.

Monteiro Bramante et al (2008)[4] conducted a study to determine the presence of Arsenic in various types of mineral trioxide aggregate (MTA) and Portland cements to verify if they comply with the ISO-recommended limit for water-based cements of 2 mg

Arsenic/kg material. An amount of 5 mL of hydrochloric acid was added to 2 g each of MTA and Portland cement to be analyzed. After 15 minutes, the material was filtered and the volume of supernatant was diluted with reagent-grade water up to 40 ml. Atomic absorption spectrophotometry readings were performed in triplicate. Results showed that all tested materials presented Arsenic in their composition. The form of Arsenic was not analyzed nor the toxicity of the Arsenic found. Only MTA-Obtura, White MTA-Angelus, and White Portland cement presented Arsenic levels below the limit set in the ISO 9917-1 standard.

Gustavo De-Deuset al (2009)[16] determined and compared the amount of Arsenic in some brands of mineral trioxide aggregate (MTA) and Portland cement. Arsenic species (As [III], As [V], and dimethylarsinic acid) were separated by high-performance liquid chromatography (HPLC) using a strong anion exchange column and converted into arsines by online HG. The instrumental coupling, HPLC-HG-AFS, was applied to 0.2 g of each cement that was prior digested in a solution of HCl, HNO3, and HBF4. Data were expressed as a part per million, and the preliminary analysis of the raw pooled data revealed a bell-shaped distribution. Statistical analysis was performed using one-way analysis of variance for multiple comparisons. In all chromatograms obtained, only type III Arsenic could be detected. The minimum amount of Arsenic was detected in samples of white MTA ProRoot (3.3 $\pm$ 10-4) and the

maximum in the samples MTA Bio Angelus (Angelus, Londrina, PR, Brazil) (8.6 ± 10-4). In the Gray MTA (Angelus), gray Pro- Root MTA (Tulsa/Dentsply, Tulsa, OK) and CP Juntalider (Brasilatex Ltda, Diadema, SP, and Brazil) did not detect any trace of Arsenic. The values of Arsenic found in CP Irajazinho (Votorantim Cimentos, Rio Branco, SP, and Brazil) and white MTA Angelus were intermediaries to minimum and maximum values. The nonparametric test Kruskal- Wallis showed statistically similar results among all cements tested ($p > 0.5$).

Woo Chang et al (2010)[9] evaluated the levels of 10 heavy metals (Arsenic, Bismuth, Cadmium, Chromium, Copper, Iron, Lead, Manganese, Nickel, and Zinc) in gray Portland cement (GPC), white Portland cement (WPC), gray MTA (GMTA), and white MTA (WMTA) were analyzed by inductively coupled plasma-atomic emission spectrometry. One gram of each material was digested with 80°C "aqua-regia" (7 mL of 60% HNO3 and 21 mL of 35% HCl), filtered, and analyzed by ICP-AES. The analysis was performed 6 times and the data were analyzed statistically. The results showed that Arsenic and lead concentrations were the highest in GPC ($P > .05$). GPC had much more of 7 heavy metals than the other 3 cements ($P > .05$). GMTA and WMTA had higher purity than GPC and WPC ($P > .05$), particularly when Arsenic content was considered.

Tsunenori Matsunaga (2010)[34] investigated whether the concentration of Arsenic (As) released from gray or white mineral trioxide aggregates (MTAs) met the requirement of the International Standards Organization (ISO) for dental cements. Sample preparations were carried out according to the ISO methods. After centrifugation of dissolved samples, As (III) concentration in the final supernatant was analyzed by a high-performance atomic absorption spectrophotometer. Results showed that As (III) concentration from both MTAs was much less than the required value (2 ppm) for dental cements regulated by the ISO. An experiment simulating pulp capping by using MTA revealed that As concentration was also below the standard value of the ISO. The As concentration in white MTA was lower than the value (10 ppb) recommended for tap water and environmental standards.

Masoud Parirokh(2010)[33] Reviewed the chemical, physical and antibacterial properties of Mineral Trioxide Aggregate. A review of the literature was performed by using electronic and hand searching methods for the chemical and physical properties and antibacterial activity of MTA from November 1993–September 2009. His results showed that MTA is composed of calcium, silica, and bismuth. It has a long setting time, high pH, and low compressive strength. It possesses some antibacterial and antifungal properties, depending on its powder to liquid ratio.

Torabinejad, et al (2010) reviewed the Leakage and Biocompatibility of Mineral Trioxide Aggregate. A review of the literature was performed by using electronic and hand-searching methods for the sealing ability and biocompatibility of MTA from November 1993–September 2009.He fond that on the basis of available evidence it appears that MTA seals well and is a biocompatible material.

Matthew Schembri, (2010)[27] analysed the Heavy Metals in Mineral Trioxide Aggregate and Portland Cement. Measurements of Arsenic, lead, and chromium in hydrated gray and white Portland cement, ProRoot MTA, and MTA Angelus were conducted with graphite furnace atomic absorption spectrophotometry after acid digestion on the hydrated material. The leaching of the metal ions from the solid material in water and simulated body fluid (SBF) was also determined. He found that all cement types showed high relative values of leached chromium compared with Arsenic and lead in both the total metal content and leached species. The gray Portland cement showed the highest total amount of metal. The white Portland and both MTAs had lower values for all the leached metal ions.

Darvell (2011)[13] reviewed an update and setting reaction of Mineral Trioxide Aggregate. The literature was searched using on-line tools, overlapping an earlier substantial review to pick up any omissions,

including that in respect of ordinary Portland cement (OPC), with which MTA shares much. The search was conducted for the period January 2005 to December 2009 using 'MTA', 'GMTA', 'WMTA', and 'mineral AND trioxide AND aggregate' as keywords, with various on-line search engines including Science Direct A generic name for this class of materials, Hydraulic Silicate Cement (HSC), is proposed, and an outline reaction scheme has been deduced. HSC has distinct advantages apparent, including sealing, sterilizing, mineralizing, dentinogenic and osteogenic capacities, which research continues to demonstrate. However, *ad hoc* modifications have little supporting justification.

Seok-Woo Chang, (2011)[10] investigate the heavy metal contents (As, Cr, Cr^{6+}, and Pb) of Ortho MTA and compare them with those of ProRoot MTA. One gram of each MTA was digested using a mixture of hydrochloric and nitric acids and filtered. The As, Cr, and Pb in the resulting filtrates were analyzed by inductively coupled plasma–optical emission spectrometry. The level of Cr6+ was measured by the methods suggested in the Korean Standard L 5221.The results showed that the concentration of As in ProRoot MTA was 1.16 ppm, but As was not detected in Ortho MTA. Cr^{6+} and Pb were not detected in either MTA. Ortho MTA contained significantly less Cr than ProRoot MTA ($P < .05$).

MATERIALS AND METHODS

MATERIALS USED

1. White Portland cement
2. White Proroot MTA(Dentsply)
3. White Angelus MTA(Angelus)
4. 2.4mol/L Hydrochloric acid
5. Simulated Body Fluid (SBF)
6. Concentrated Nitric acid
7. Distilled water

ARMAMENTARIUM USED

1. Glass Slab
2. Spatula
3. Weighing Balance
4. Sterile conical flask 150ml
5. Measuring jar 200ml
6. Motor and Pestle
7. Sterile Sterilization pouch
8. Incubator
9. Micropipette

APPARATUS USED

1. Graphite Atomic Absorption Spectrophotometer

METHODOLOGY

Determination of Acid-soluble Ion Content

PREPARATION OF MTA AND PORTLAND CEMENT

Two grams of Portland cement, Proroot MTA, and Angelus MTA were weighed in a weighing balance. Portland cement, Proroot MTA and Angelus MTA were mixed in a sterile glass slab with a spatula in a water powder ratio as recommended by the manufacturer. The mixed Portland cement and Proroot MTA and Angelus MTA were made into thin disc and then placed in a separate sterile sterilization pouch individually and then incubated in an incubator at 37°C and 100% humidity for 24hours.

ACID DIGESTION OF HYDRATED MATERIAL

After 24hours the set Portland cement and Proroot MTA and Angelus MTA were taken out and then placed individually in a Motor and Pestle and then crushed into a fine powder. The powdered material was placed individually in a 150ml conical flask to which freshly prepared 2.4 mol/L of Hydrochloric acid is added. The slurry was stirred well individually and is tightly capped and allowed to stand for 16 hours. After 16hours the solution was taken out and placed in a centrifuge and centrifuged for 10min.The supernatant solution was then

transferred to an acid washed sterile plastic tubes and is stopperred. The Samples have been labeled as PC-001 for Portland cement, PR-002 for Proroot MTA and AN-003 for Angelus MTA.

GRAPHITE ABSORPTION SPECTROPHOTOMETER

The graphite absorption spectrometer used in this study is Shimadzu Atomic Absorption Spectrophotometer AA-6800.This device was equipped with ASC-6100 Auto Sampler, a Shimadzu graphite furnace pyrocoated tube with forked platform 90® contact (Shimadzu Scientific Instruments, Tokyo, Japan), a Julabo F12 cooling system (Julabo, Vista, CA), a Pb Cathodeon Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), a Cr Cathodeon Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), and As Photon Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), and using an argon atmosphere for the furnace at TA(Total Analytics) Labs Pvt LTD.Chennai

The samples were then placed in the platform for the detection of As, Pb and Cr. The reading was transferred to the computer which delivers the readings in Parts per Million (ppm).

DETERMINATION OF ION RELEASE IN SOLUTION

600 milligrams of Portland cement, Proroot MTA, and Angelus MTA were weighed in a weighing balance. Portland cement and Proroot MTA and Angelus MTA were mixed in a sterile glass slab with a spatula in a water powder ratio as recommended by the manufacturer. The mixed Portland cement and both the MTAs were transferred to a acid washed molds 20mm diameter and then placed in a separate sterile sterilization pouch individually and then incubated in a incubator at 37°C and 100% humidity for 24hours.

Six cement discs were prepared for each type of cement. Three cement discs from each type were placed in 25ml of distilled water in a 150ml conical flask. The remaining three cement disc from each type was placed in 25ml of simulated body fluid in a 150ml conical flask.

COMPOSITION OF SIMULATED BODY FLUID

It is prepared freshly by mixing the reagents with distilled water.

REAGENT	**WEIGHT% for 1000ml**
NaCl	8.035g
NaHCO3	0.355g
KCL	0.225g
$K_2HPO_4.3H_2O$	0.231g
$MgCL_2.6H_2O$	0.311g
1.0M-HCL	39ml
$CaCL_2$	0.292g
Na_2SO_4	0.072g
Tris	6.118
1.0M-HCL	0-5m

The solution was prepared using the above mentioned composition in Biochemistry department of Ragas Dental College, Chennai.

The samples were then incubated at 37°C and 100% humidity. The Simulated body fluid and Distilled water were changed at 24hours, 7 days. The leachate solution from each sample is spiked with freshly prepared 250µL of concentrated nitric acid and placed in a refrigerator until further use.

The graphite absorption spectrometer used in this study is Shimadzu Atomic Absorption Spectrophotometer AA-6800.This device was equipped with ASC-6100 Auto Sampler, a Shimadzu graphite furnace pyrocoated tube with forked platform 90º contact (Shimadzu Scientific Instruments, Tokyo, Japan), a Julabo F12 cooling system (Julabo, Vista, CA), a Pb Cathode on Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), a Cr Cathode on Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), and As Photon Hallow Cathode Lamp (Perkin Elmer, Wellesley, MA), and using an argon atmosphere for the furnace.

The samples were then placed in the platform for the detection of As, Pb and Cr. The reading was transferred to the computer which delivers the readings in Parts per Million (ppm).

PHOTOGRAPHS

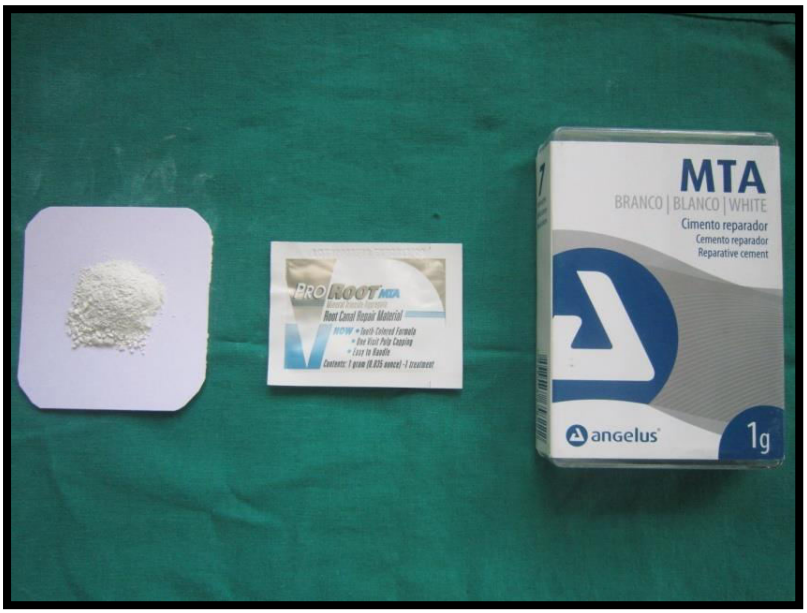


Figure - 1: Portland cement, Proroot MTA and Angelus MTA

Figure - 2: CONC HCL, Distilled water, CONC HNO_3

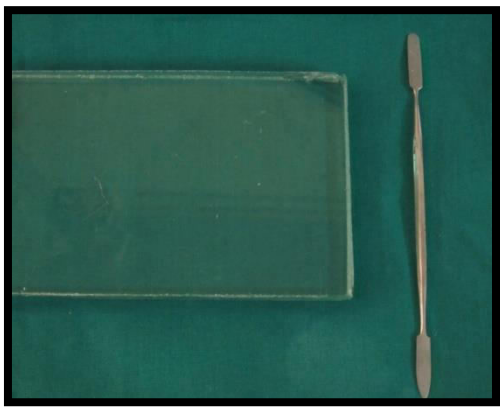


Figure - 3: Glass slab & spatula

Figure - 4: Sterilization pouch

Figure - 5: Motor and pestle

Figure - 6: Weighing balance

Figure - 7: Measuring jar

Figure - 8: Incubator

Figure - 9: Micro pipette

Figure - 10: Mixed cements

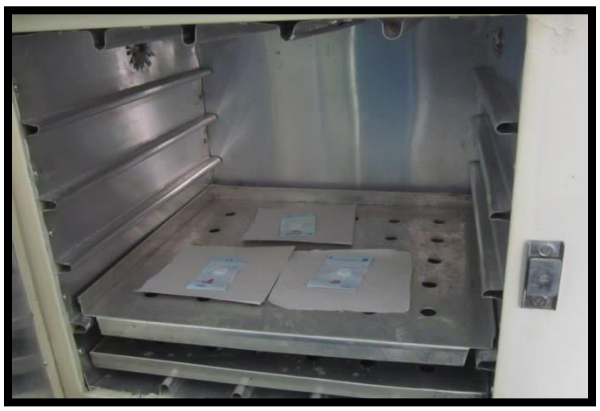


Figure - 11: Samples in incubator

Figure - 12: Digested samples

Figure - 13: Graphite Atomic Absorption Spectrophotometer

RESULTS

The results were obtained in mg/Kg ie. Parts per million. Proroot MTA, Angelus MTA and Portland cement exhibited a high level of Arsenic and Chromium than the lead. The Highest level of chromium is for Angelus MTA Cement (50.27mg/Kg) followed by Portland cement (48.79mg/Kg) which is followed by Proroot MTA (37.33mg/Kg).

Portland cement showed a higher amount of Arsenic (58.33mg/Kg) followed by Angelus MTA (43.12mg/kg) and Proroot MTA (41.22mg/Kg). The acid-soluble arsenic level of Proroot MTA, Angelus MTA and Portland Cements was higher than the level suggested by ISO 9917-1 (2007) specifications for dental materials, whereas for the lead it's lower ie. Portland cement (3.45mg/Kg), Proroot MTA (1.68mg/Kg), Angelus MTA (2.43mg/Kg). (Table 1)

The release of metal ion in water and simulated body fluid showed the same trend as the total metal concentration with chromium being the highest followed by arsenic and lead. All the samples tested showed an initial high release of metal ions in the first 24 hours in both water and simulated body

fluid. The release in SBF was higher than in water for all the metal ions tested in the first 24hours of exposure to the solution. (Table 2, Table 3)

Portland cement released the highest level of Chromium, Arsenic and lead than ProRoot MTA and Angelus MTA. Portland cement and MTA Angelus released similar levels of arsenic, which was higher than that released by ProRoot MTA in both water and simulated body fluid. The release in SBF was higher than in water for all the metal ions tested in the first 24 hours of exposure to the solution ($p < 0.001$).

In the 7 days period the detection of Arsenic, lead and Chromium is below the recommended ISO level, with Chromium being the highest followed by Arsenic and lead. Arsenic in the 24 hours sample and 7 days sample also showed the highest release of metal ions in simulated body fluid than in water. Clinically this is very important because it indicates the long term problems of continous release of metal ion in the body fluid are low.

To summarize the results

Total Ion Content

1. The heavy metal content of Portland cement were Arsenic-58.33 mg/Kg, Lead-3.4 mg/Kg,Chromim-48.79 mg/Kg.
2. The heavy metal content of Proroot MTA were Arsenic-41.22 mg/Kg, Lead-1.68 mg/Kg, Chromim-37.33 mg/Kg.
3. The heavy metal content of Angelus MTA were Arsenic-43.12 mg/Kg, Lead-2.43 mg/Kg, Chromim-50.27 mg/Kg.

Metal Ion Release after 24 hrs exposure time

1. The heavy metal ion release of Portland cement in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	1.103	1.236
Pb	0.326	0.47
Cr	4.43	4.88

2. The heavy metal ion release of Proroot MTA in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	0.84	1.07
Pb	0.70	0.14
Cr	2.72	3.06

3. The heavy metal ion release of Angelus MTA in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	1.03	1.16
Pb	0.10	0.18
Cr	2.12	2.93

Metal Ion release after 7 Days exposure time

1. The heavy metal ion release of Portland cement in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	0.97	0.98
Pb	0.15	0.30
Cr	1.07	1.70

2. The heavy metal ion release of Proroot MTA in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	0.64	0.69
Pb	0.05	0.08
Cr	1.16	1.98

3. The heavy metal ion release of Angelus MTA in water and SBF were

	Water(mg/Kg)	SBF(mg/Kg)
As	0.77	0.82
Pb	0.05	0.09
Cr	1.68	1.48

TABLE 1: TOTAL METAL ION CONTENT (mg/Kg)

	ARSENIC	**LEAD**	**CHROMIUM**
Portland Cement	58.33	3.4	48.79
Proroot MTA	41.22	1.68	37.33
Angelus MTA	43.12	2.43	50.27

TABLE 2: METAL ION LEACH AFTER ONE DAY IN WATER (mg/Kg)

	ARSENIC	LEAD	CHROMIUM
PCIWA	1.09	0.32	4.39
PCIWB	1.12	0.35	4.48
PCIWC	1.10	0.31	4.42
PRIWA	0.89	0.08	2.68
PRIWB	0.79	0.07	2.76
PRIWC	0.84	0.06	2.72
ANIWA	1.01	0.09	2.09
ANIWB	1.06	0.10	2.16
ANIWC	1.04	0.11	2.12

TABLE 3: METAL ION LEACH AFTER ONE DAY IN SBF (mg/Kg)

	As	Pb	Cr
PCISA	1.27	0.48	4.91
PCISB	1.20	0.46	4.86
PCISC	1.24	0.47	4.89
PRISA	1.06	0.12	3.02
PRISB	1.09	0.16	3.09
PRISC	1.07	0.14	3.07
ANISA	1.19	0.18	2.98
ANISB	1.14	0.19	2.89
ANISC	1.16	0.17	2.97

TABLE 4: METAL ION LEACH AFTER SEVEN DAYS IN WATER (mg/Kg)

	As	Pb	Cr
PC7WA	0.99	0.16	1.09
PC7WB	0.96	0.14	1.06
PC7WC	0.97	0.15	1.08
PR7WA	0.63	0.05	1.14
PR7WB	0.66	0.05	1.19
PR7WC	0.65	0.06	1.16
AN7WA	0.78	0.06	1.71
AN7WB	0.79	0.04	1.66
AN7WC	0.76	0.05	1.69

TABLE 5: METAL ION LEACH AFTER SEVEN DAYS IN SBF (mg/Kg)

	As	Pb	Cr
PC7SA	0.99	0.30	1.68
PC7SB	0.99	0.28	1.72
PC7SC	0.96	0.32	1.70
PR7SA	0.68	0.07	2.01
PR7SB	0.69	0.09	1.96
PR7SC	0.70	0.08	1.99
AN7SA	0.84	0.09	1.52
AN7SB	0.81	1.00	1.47
AN7SC	0.82	0.09	1.45SS

ACID SOLUBLE ION CONTENT

Two grams of Portland cement, Proroot MTA and Angelus MTA were mixed with water or the liquid provided

↓

The mixture cured in oven at 37^0 C & 100% humidity for 24 hours

↓

After 24 hours, the material is made into fine powder with agate motor and pestle

↓

The powdered material is placed in 150ml conical flask and 50ml of 2.4 mol/L of HCL is added and allowed to stand for 16 hours

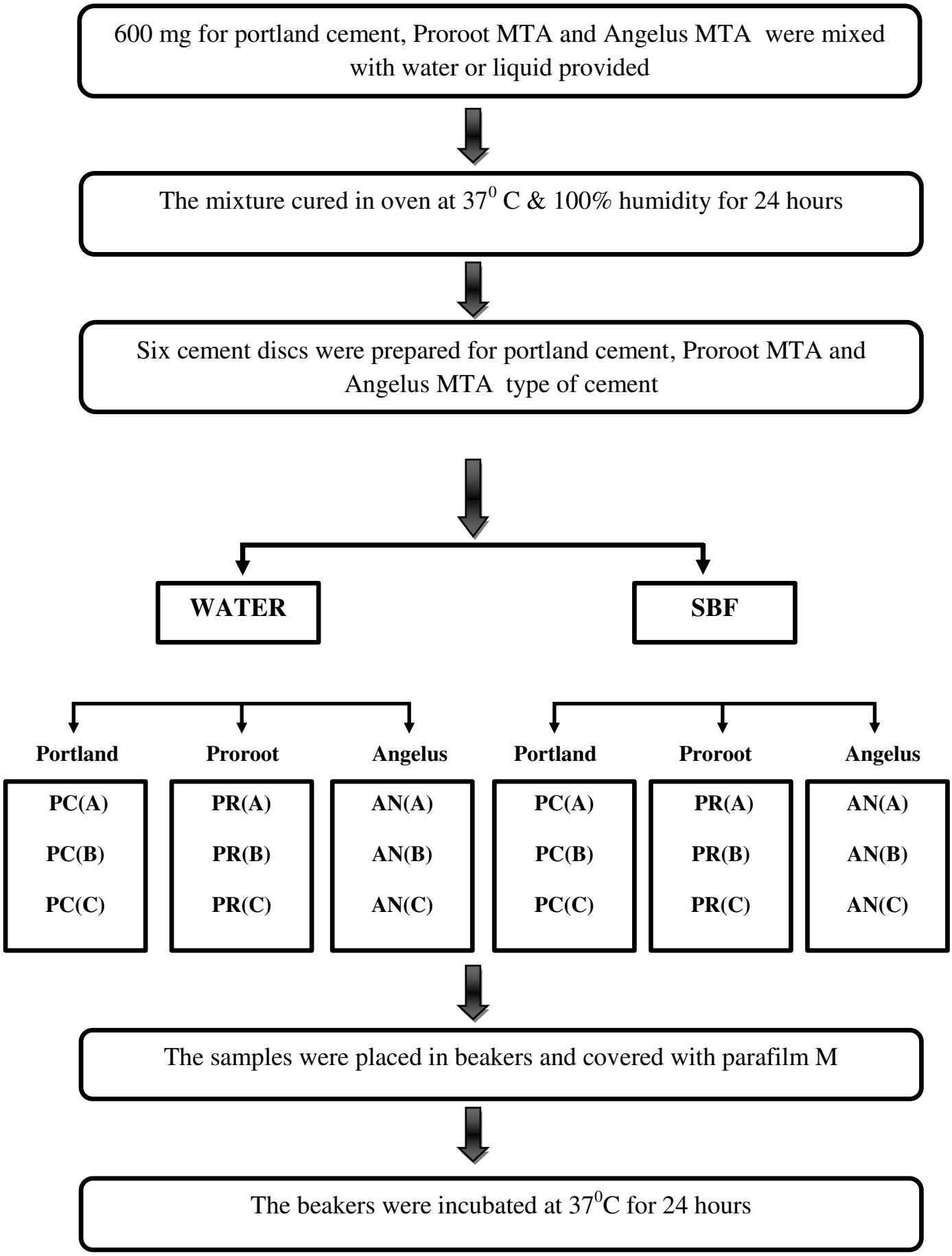
↓

After 16 hours, the solution centrifuged for 10minutes

↓

The solution is then placed in graphite furnace atomic absorption spectrophotometer for the detection of Arsenic, Lead and Chromium

DETERMINATION OF ION RELEASE



The solutions was changed after 24 hours and seven days

The leachates was spiked with 250µ L of concentrated nitric acid

The solution were then placed in graphite furnace atomic absorption spectrophotometer for the detection of Arsenic, Lead and Chromium levels

DISCUSSION

Over the period of time, there has been a continuous search for dental materials that presents good physicochemical and biologic properties. Several retrograde or orthograde root filling materials have been proposed and suggested, such as silver amalgam, gutta-percha, zinc oxide– eugenol cements (IRM, Super EBA, Rickert), glass ionomer, composite resins, calcium hydroxide cements (Sealapex, Sealer, and more recently the Mineral Trioxide Aggregate (MTA). Root end surgery has historically served as the most conservative final effort to resolve periradicular inflammation after the non-surgical therapy has failed or not possible.

Mineral trioxide aggregate MTA has emerged as a popular root-end filling material because of its biocompatibility and superior sealing ability. MTA is an endodontic biomaterial originally used for the purpose of root end filling material over time its clinical application have expanded to vital pulp therapy, including pulpotomy, apexification, surgical and non-surgical perforation repair. (MTA) has almost 15years of clinical experimental success with an ample variety of applications. In addition to its good sealing ability and

biocompatibility, MTA was found to facilitate the over growth of cementum, regeneration of the periodontal ligament and formation of bone. It was developed at Loma Linda University by Torabinajed in the year 1993,

Mineral trioxide aggregate (MTA) consist of 50-75% wt calcium and 15-25% silicon dioxide. These two components together comprise 70-95% of the cement. When these raw materials are blended they produce tricalcium silicate, dicalcium silicate, dicalcium alumina and tetra calcium alumino ferrite which on addition of water hydrates to form silicate hydrate gel. Torabinejad et al.[31] developed the original product (gray MTA). The main constituents of this material were calcium silicate ($CaSi0_4$), bismuth oxide (Bi_20_3), calcium carbonate ($CaC0_3$), calcium sulfate (CaS04), and calcium aluminate $(CaAl_20_4)$[17]. MTA was originally marketed as grey coloured preparation and has been associated with occasional staining of the teeth. Therefore a white MTA material has been recently developed to overcome this concern.

Different brands of MTAs available in the market are Proroot MTA (Dentsply), (Grey and White).MTA Angelus (Grey and White). Chemically two different type of MTA are

available ie. Grey MTA (GMTA) and White MTA (WMTA). The main difference in chemical composition between GMTA and WMTA is a significant decrease in the amount of iron and other metallic elements in the later.

It has been found that Portland cement type 1 is the main component of MTA with addition of bismuth oxide at 4:1 ratio to provide radiopacity.[32] Great similarity between MTA and Portland cement has been found in respect to the composition of the basic elements, the antimicrobial action, and the biological properties. Portland cement is the worldwide one of the most widely employed materials in construction industry. This material is manufactured by a clinkering process or partial fusion of raw materials. This process includes limestone decarbonization at 400 to 600°C; formation of dicalcium silicate, tricalcium aluminate, and tricalcium aluminoferrite between 800 and 1200°C; and production of tricalcium silicate at 1400°C by the reaction of dicalcium silicate with free lime[18] forming clinker. Thus the material may contain some heavy metals and contaminants like Arsenic (As), Lead (Pb), Chromium (Cr).

The heavy metals are incorporated from the substitution of primary fuels to reduce costs with alternate fuels, which are usually wastes, and also by the utilization of wastes, an alternative inorganic raw material together with the limestone and shale [27]. Waste used as an inorganic raw material or used as a secondary fuel may contain widely varying metal concentrations compared with raw materials and regular fuels. Trace elements found in wastes that are used in cement production may either be transferred to the cement or emitted with exhaust gases into the environment.

The original MTA patent 5,769,638[32] indicated the use of American Society for Testing Materials type 1.

The aim of this present study was to investigate the levels of Heavy metal contents present in Portland cement, Proroot MTA, and Angelus MTA.

The materials used in this study are Portland cement, Portland MTA Cement (Dentsply, Tulsa Dental Products, Tulsa, MTA Angelus (Angelus Solucoes Odontologicas, Londrina, Brazil

The total metal content was determined by the ISO 9917-1 (2007)[5] method. Two grams of the Portland cements and MTAs were mixed with water or the liquid provided. They were placed in separate plastic bags, and flattened to produce a very thin disc. The mixture was then allowed to cure in an oven at 37ºC and 100% humidity for 24 hours. The disc was crushed to a very fine powder in an agate mortar and pestle. Two grams of the powdered material were placed in a 150-mL conical flask and 50 mL of 2.4 mol/L of hydrochloric acid were added. The slurry was swirled well, lightly stoppered, and allowed to stand for 16 hours.

The solution was then centrifuged in a tube for 10 minutes. The supernatant was transferred to a stoppered acid-washed plastic tube. The arsenic, lead, and chromium content were measured using a graphite furnace atomic absorption spectrophotometer (GFAAS).

Other techniques that could have been used for the determination of arsenic, chromium, and lead include gravimetry, colorimetry, and inductively coupled plasma. GFAAS was chosen over other techniques because it did not require additional modification of the digested sample, which

would enhance the chances of contamination, GFAAS possesses high degree of sensitivity and precision.[27]

Graphite furnace atomic absorption spectrophotometry (GFAAS) is an analytical technique designed to perform the quantitative analysis of metals in a wide variety of samples. In comparison to standard flame atomic emission or flame atomic absorption methods for the determination of metals at the trace and ultra-trace level.

A series of three heating steps are usually then applied to the sample contained in the graphite furnace. The first is the ***drying stage***, designed to gently evaporate the solvent from the liquid sample without splattering the sample. Following the drying stage, the temperature of the furnace is increased, often in the range of 400-800 ° C for a period of time called the ***ashing (or charring)*** stage. During this stage organic components (fats and oils) in the matrix can be charred, the composition of the sample can be changed chemically, or high boiling volatile components can be removed. The impact of this stage is one which further simplifies the sample matrix to help reduce interferences and make calibration easier.

Finally, the ***atomization stage*** is applied. In this stage, the temperature of the furnace is very rapidly raised to temperatures often as high as 2700 ° C, effectively volatilizing the remaining components on the rod wall The actual absorbance measurement that occurs during this stage by using a hollow cathode lamp shined through the furnace, creating the actual transmittance measurement that is common to all forms of spectrophotometry. A signal trace of absorbance versus time is the measurement that is used for quantitative purposes. The trace characteristically shows a transient absorbance signal as a function of time.[28]

Digestion of the sample is required because the determination of metals using atomic absorption spectroscopy (AAS) requires that the metals are converted to soluble ions.

600 milligrams of Portland cement, Proroot MTA, and Angelus MTA are weighed in a weighing table (Fig 6). Portland cement and both the MTAs are mixed in a sterile glass slab with a spatula (Fig 3) in a water powder ratio as recommended by the manufacturer. The mixed Portland cement and both the MTAs are transferred to a acid washed molds 20mm diameter and then placed in a separate sterile sterilization pouch

individually and then incubated in a incubator at 37°C and 100% humidity for 24hours.

Six cement discs were prepared for each type of cement. Three cement discs from each type were placed in 25ml of distilled water in a 150ml conical flask. The remaining three cement disc from each type was placed in 25ml of simulated body fluid in a 150ml conical flask. The samples were then incubated at 37°C and 100% humidity. The Simulated body fluid and Distilled water were changed at 24hours, 7 days. The leachate solution from each sample is spiked with freshly prepared 250µL of concentrated nitric acid and placed in a refrigerator until further use.

SIMULATED BODY FLUID is an acellular fluid that has inorganic ion concentration similar to those of human extracellular fluid which can be easily prepared by dissolving reagent in pure water. This was first introduced by kokubo et al in 1990 at Japan. This fluid was developed to reproduce the formation of apatite on the surface of the bioactive materials invitro, and also used as coating of apatite on various materials under biometric condition. The simulated body fluid is

abbreviated as SBF or kokubo solution, and can be adjusted to a PH 7.4.

Hydration of the MTA powder produces a colloidal gel that solidifies into a hard structure consisting of discrete crystals in an amorphous matrix. The crystals are composed of calcium oxide, and the amorphous region is composed of 33% calcium, 49% phosphate, 2% carbon, 3% chloride, and 6% silica.[3]MTA, was mainly composed of **tri and di- calcium silicates**. These silicates undergo hydrolysis producing calcium hydroxide and a less basic calcium silicate gel with the approximate composition $C_3S_2H_3$ is formed. The resulting hydrate gel is poorly crystallized and produces a porous solid, which may be defined as rigid gel. The silicate hydrate gel has typical calcium to silica ratio of approximately 1.7 which is lower than the 3;1 ratio in the tri calcium silicate. The excess calcium is precipitated as CaOH, makes the cement highly alkaline (12.5)[8] the aluminate reacts with water to form ettringite, and monosulphate in lower levels.

The pH has been reported to be approximately 9.5 at 168 hours after mixing; The setting and the subsequent leakage of MTA are not affected by the presence of blood. Holland et a1.

Theorized that the tricalcium oxide in MTA reacts with tissue fluids to form calcium hydroxide, resulting in hard-tissue formation.[19]

ISO 9917-1 (2007)[5] specifies a maximum value of 2 mg/kg and 100 mg/kg for acid-soluble arsenic and lead, respectively, for restorative dental materials.

Arsenic is a metallic element with atomic number 33 and was discovered in 1250. Found in water, soil, and air from natural and anthropogenic sources. It exists in inorganic and organic forms and in different oxidation states (−3, 0, +3, +5)[2]

Lead serves no useful purpose in the human body, and its presence can cause toxic effects, regardless of the exposure pathway. Proposed mechanisms for the toxicity of lead involve fundamental biochemical processes including its ability to inhibit or mimic the actions of calcium (which can affect calcium-dependent or related processes) and interaction with proteins. It must be emphasized that lead levels at which health effects have been observed are constantly being revised, and that, for children especially, there may be a no threshold for the development of detrimental effects[26]

Chromium (VI) can act as an oxidant directly on the skin surface or it can be absorbed through the skin, especially if the skin surface is damaged. Once absorbed into the blood system, there are various antioxidants that act as reducing agents, such as glutathione and ascorbate, which rapidly reduce chromium (VI) to chromium (III). The affected person might experience an upset stomach or sometimes stomach ulcers can develop too. Chromium toxicity can cause respiratory problems such as difficulty in breathing and coughing and may lead to bronchitis or asthma[22]

Since toxicity is based on the effect that a toxicant produces at a target site within an organism, establishing the relationship between the concentration of the substance at the target site and the subsequent toxic effect can provide a tool for predicting toxicity. This is the primary toxicological principle generally referred to as ‘‘dose response’’ or ‘‘concentration response’’ in which the response of an organism is proportional to the dose or concentration of the substance at the target site. In many cases the target site is unknown, or measurement of the substance at the site is not possible.[23]

The total chromium, arsenic, and lead levels of white Portland cement were shown to be higher than the two MTAs tested. Arsenic levels were below those of chromium in all the three cements under investigation. This is because Arsenic in various oxidation states ie As_2o_5, As_2o_3, As_2s_3 decomposes at 315ºC,465ºC,707ºC respectively. Since the raw materials are heated in a rotary kiln at temperature ranging from 1,250ºCto 1,500ºC most of the Arsenic states get decomposed [27]. High concentration of arsenic have been found in the all the cement. Lead was the least abundant metal ion of all the three cement types. It is an alkaline byproduct of cement manufacture.

The high amount of all heavy metals in Portland cement may be the results from the grinding of a product called clinker obtained by cooking a mixture of dosed and homogenized lime and clay until incipient fusion in such a way that all substances only combine with the clayish compounds, without resulting in free lime in harmful amounts after cooking.

The total levels of Arsenic is more than the recommended ISO9917-17(2007)[5] in all the three cements tested. From the results of the present study, both MTAs showed a similar level

of contamination as white Portland cement. Arsenic is the poison of choice for many murders in fiction and in reality.

The total Arsenic content detected in previous studies were less than this present study[4,9,15]. This may be attribute to the difference in the digestion method of the sample. In that investigative study, method of digesting the sample is as not equivalent to ISO9917-1. Although using the same reagents suggested by the ISO standard, the concentrations of the acids used were lower, and the filtering was performed after 15 minutes as opposed to the 16 hours suggested by the standard ISO method. The lower concentrations of acid and the shorter time duration may have prevented the complete dissociation of the metals ions, thus explaining the lower quantities of arsenic detected in that study. Furthermore, the additional filtration step could have resulted in losses of arsenic because of its volatility[4].

Lot of methods in literature has been proposed for the digestion of the sample[4, 9, 15]. Each differs in the concentration of the reagent, time to digest and the investigating device. To minimize variability, one has to use a digestion method with the

minimum reagent and the optimum conditions, the most important of which being the temperature.

On the other hand the release of metal ions in water and simulated body fluid were almost equal or less than the ISO standard recommendations. The metal ion release from the both the liquids showed the same trend as the total metal ion concentration. Portland cement showed the highest release of Chromium, Arsenic and Lead compared to Proroot MTA and Angelus MTA. The results obtained show that chromium was released in larger quantities followed by arsenic and lead. The release of metal ions in 24 hour is higher than the 7 days period indicating the initial high release of metal ions which gradually declines.

In all the tested samples, the metal ion release was comparatively more in simulated body fluid than in water. This may be attributed to the presence of trisma buffer which reduces the pH of the solution thus enhancing further release of metal ions.

Chromium VI is found to be more toxic and carcinogenic than other oxidation species of Chromium. The Portland cement

certification had a limit of 0.4 ppm for Cr (VI). In this present study only total Chromium amount has been detected because for the detection of oxidation states of chromium, the samples should undergo separation stage using High performance liquid chromatography[27]

The level of Arsenic was more in all the samples than the recommended ISO 9917-1.The acid soluble arsenic content detected from the samples in this study indicates the total amount of Arsenic. But it is always not necessary that all the trace elements will release from the sample as was shown in this study. The MTAs were all based on Portland cement which is basically alkaline in nature. The metal ion release was dependent on the medium. The reduction in pH and stabilization of the solution pH with a buffer resulted in the release of more trace metals in the simulated body fluid than in water.

An interesting aspect in the results of the present study was the detectable amount of Arsenic found in the Proroot MTA and Angelus MTA. From these findings, it can be proved that MTA formulations are not totally free of undesirable contaminant substances as claimed in the product. The Arsenic levels in the two MTA products were found to be 20 times more

than the limits specified by the ISO Standard(9917-1). It is not known if the Arsenic released in solution is detrimental to the health of the host because the ISO standards only specify the limits for the total Arsenic content and not for the released species. It is prudent that future investigations should explore, if these contaminants particularly Arsenic can contribute to specific malfunction in the human body due to a cumulative effect. Cell toxicity assays can also be performed to simulate the effects of such materials in the natural environment.

SUMMARY

The purpose of this study was to evaluate the acid soluble Arsenic, Lead and Chromium content of Portland cement, Proroot MTA and Angelus MTA and also to find the metal ion leach in water and simulated body fluid at 24hrs and 7 days.

The total metal was determined by the ISO 9917-1 (2007) method. Two grams of the Portland cements and MTAs were mixed with water or the liquid provided, placed in separate plastic bags, and flattened out to produce a very thin disc. The mixture was then allowed to cure in an oven at 37_C and 100% humidity for 24 hours. The disc was crushed to a very fine powder in an agate mortar and pestle. Two grams of the powdered material were placed in a 150-mL conical flask and 50 ml of 2.4 mol/L of hydrochloric acid were added. The slurry was swirled well, lightly stoppered, and allowed to stand for 16 hours

The solution was then centrifuged in a tube for 10 minutes. The supernatant was transferred to a stoppered acid-washed plastic tube. The arsenic, lead, and chromium content were measured using a graphite furnace atomic absorption

spectrophotometer 600mg of Portland cement and both the MTAs are mixed with the water and liquid provided by the manufacturer Cured at 37 C for 24 hrs six cement disc are made for each cement type. Three are placed in water and other three in simulated body fluid. The solutions were changed at 24hs and 7 days. The amount of Arsenic, lead and chromium leached was observed using graphite furnace atomic absorption spectrophotometer.

The data's were obtained in Parts per Million (PPM). Analysis of variance with p = 0.05 and the One way Anova test were used to perform multiple comparison tests. The results were tabulated.

CONCLUSION

Under the limitations of this present study it can be concluded that:

1. The Heavy Metal (Arsenic,) content in Portland cement, Proroot MTA, Angelus MTA were higher than the recommended level (ISO 9917-1).
2. Lead content was found to be the least in all the cement types tested.
3. Chromium was found to be more in Portland Cement, Proroot MTA and Angelus MTA than arsenic and lead.
4. Higher amount of leaching of Heavy metals (As, Pb, Cr) were found in Portland cement.
5. Proroot MTA Showed the least release of Heavy metal ions followed by Angelus MTA.

Further systemic long term studies can be undertaken to investigate the potential toxicity of Heavy metals present in Mineral Trioxide Aggregate.

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