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Keywords: allelopathy, seed germination, tomatoes, okra, Amaranthus.

Abstract: In a search for effective methods for controlling seed germination and growth, bio-oils were produced from pyrolysis of bunches of empty palm fruit at different temperatures. Physicochemical chemical characteristics of the bio-oils were evaluated using standard analytical procedures. Allelopathic activities of the bio-oils were evaluated against three selected seeds (tomatoes, okra and Amaranthus). The percentage germination inhibition was calculated for each seed after 72 h of germination. The yields of the bio-oils are 22.07%, 35.13% and 37.47% at 400 °C, 500 °C, and 600 °C, respectively. The bio-oils are acidic and contain compounds such as phenols, phenol derivatives, alkanes, and organic acids. The results revealed that the empty palm fruit bunches' bio-oils have inhibitory effects on the three selected seeds. The percentage seed germination decreased with increasing concentration of the bio-oils while the inhibitory effect of the bio-oils on seedling growth increased significantly with increasing concentration of the empty palm fruit bunches' bio-oils. The bio-oils obtained at different pyrolytic temperatures showed appreciable allelopathic activities.

1 Introduction

The hazardous environmental issues arising from the use of synthetic herbicides, which started in 1960 after the enormous labour and time consuming associated with traditional ways of controlling weeds, have become unbearable [1,2]. Consequently, the search for alternative ways of controlling weeds, with less detrimental effects, are needed. Among the numerous alternative ways of controlling weed include the usage of allelochemicals [3]. Unlike synthetic herbicides, plant extracts and essential oil are environmentally friendly [4].

Bio-oil is a dark-brown condensable liquid of pyrolysis. Bio-oils are characterized with unpleasant smoky odour, high viscosity and varying quantities of water, depending on the types of biomass. Chemically, bio-oils are complex oxygenated organic compounds that are reformed through catalytic cracking into bio-fuels, which serve as substitutes for liquid fossil fuels in some applications [5]. Bio-oils can be used to produce flavours for food industries and herbicides for agricultural purposes. Other researches have

shown the potential application of bio-oils as renewable resins [6].

Allelopathy is a process that involves secondary metabolites from microorganisms or plants to produce inhibitory effects on other plants, insects or microorganisms by influencing their growth and development. It is regarded as the chemical inhibition of one plant (or other organisms) by another plant due to the release of substances, which can act as germination or growth inhibitors, into the environment [7,8]. Allelopathic potential of some plants has been reported to inhibit *Medicago polymorpha* [9].

Allelochemicals contribute to weed management by releasing toxic chemicals into the soil which suppressed the germination of weed's seed [10,11]. The formation of secondary metabolites depends on the plant family as well as the climatic conditions [12]. The inhibitory effects of allelochemicals depend on their concentration and usage [13]. Oracz et al. [14] and Bogatek et al. [15] reported that the allelopathic extracts from sunflower influenced the

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antioxidant system in targeted plants, thereby damaged plant cellular and allowed the cell membrane to be swollen and inhibited the germination of targeted plant which led to loss of seed's vigour. Allelopathic properties are not limited to crude extracts of plants, therefore, research must be extended to other forms of plants' extracts such as plant's oil or biomass oil. Jamil et al. [16] and Farooq et al. [17] reported the importance of allelopathic water extract potential of crop plants in controlling weeds. Oracz et al. [14] reported positive allelopathic potential of polyunsaturated fatty acids against plant cell membrane. In view of this, the present study was carried out to evaluate the inhibitory effect of the bio-oils obtained from slow pyrolysis of the bunches of empty palm fruits against germination and seedling growth of three selected vegetable seeds.

2 Methodology

2.1 Plant sample

Empty palm fruit bunches (*Elaeis guineensis* J.) were obtained from the Teaching and Research Farm of The Federal University of Technology, Akure, Nigeria. The samples were reduced or cut into smaller chips that could be fed into the reactor canister. The biomass was sun-dried for approximately 8 weeks before it was brought to the laboratory and oven-dried at 105 °C for 12 h. The samples were then stored in sample bags prior to pyrolysis.

2.2 Pyrolysis of empty palm fruit bunches

The pyrolysis of the dried biomass feedstock for bio-oil production was carried out using a fixed bed slow pyrolysis at three different temperatures (400, 500 and 600 °C) as earlier reported [18,19].

A 1.0 kg of empty palm fruit bunches biomass was packed into the pyrolysis canister and placed in the muffle furnace (pyrolyser) for heating. The temperatures of the outer chamber of the furnace were separately set at 450 °C, 550 °C, and 650 °C (furnace temperature) and those of the inner chamber were correspondingly set at 400 °C, 500 °C, and 600 °C (canister temperature). The volatiles escaped from the canister through the hole in the lid when the furnace temperature reached 250 °C, and as the temperature of the furnace was increased, the volatiles escaped continuously and were condensed as pyrolyzed liquid condensates (bio-oil and bio-tar) into reagent bottle immersed in water bath as a receiver in the condensing unit. The non-condensable syngas was allowed to escape into the atmosphere after passing through a water washer. The pyrolytic temperature was maintained until the required experimental temperature was reached, and the reaction time was recorded. The biochar and liquid condensates produced were allowed to cool overnight, removed, weighed and stored in airtight containers while the bio-oil was stored in a clean bottle for further analysis and experiment. The same procedures were used for all the

pyrolytic temperatures. All the pyrolytic reactions were carried out in triplicate.

2.3 Characterization of bio-oils

The Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) and American Society for Testing Materials–Miscellaneous Materials (ASTM D-2866) methods were used to investigate the physicochemical analyses of the empty palm fruit bunches' bio-oils. The bio-oils were also characterized using Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Mass Spectroscopy (GC-MS).

2.3.1 Treatment

From the bio-oils, three treatments at different concentrations of 1.0%, 2.5%, and 5.0% (w/v) were prepared for each of the temperature, controls were also prepared with distilled water for each bio-oil concentration.

2.3.2 Tested seeds treatment

The seeds were germ screened according to a method described by El-Darier [20]. Okra, Tomatoes and Amaranthus seeds were thoroughly washed, first with tap water and subsequently with distilled water. They were surface-sterilized by soaking for 90 – 300 seconds in a solution of 0.5% sodium hypochlorite, and thereafter rinsed separately 3 – 5 times with distilled water [21].

2.4 In vitro biotest

2.4.1 Seed germination test

The seed germination tests were carried out according to the method reported by Sikolia and Ayuma [22] and Suwal et al. [23] with a little modification. Viable seeds were obtained from Agricultural Development Project (ADP), Akure, Nigeria. Ten (10) uniform seeds of Okra, Tomatoes and Amaranthus were placed in separate Petri dishes lined with a double layer of 9 cm Whatman filter paper, 10 mL of each of the various prepared concentrations (5.0, 2.5 and 1.0% w/v) were added to each of the Petri dish containing filter paper to make it moistened. Ten seeds were counted and placed in each plate and covered. The Petri dishes were then placed inside a dark cupboard. Controls were similarly prepared with distilled water. Triplicates Petri dishes were prepared differently for each concentration as well as each bio-oil produced at different temperatures for the three selected seeds. The percentage germination (%G) of each of the seeds were calculated by dividing the total number of seeds that germinated after 72 h, in each treatment, by the number of seeds sowed multiplied by 100. The percentage germination inhibitions were calculated by comparing with control using Equation 1.

$$\% \text{ inhibition} = \frac{Y-X}{Y} \times 100 \quad (1)$$

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where Y is the number of seeds germination in control, and X is the number of seeds germination in treatment.

2.5 Seedling growth test

The tests on seedling growth were performed according to methods reported by Casimiro et al. [24] and Suwal et al. [23], with slight modifications. Healthy seeds were collected from Agricultural Development Project (ADP) in Akure, Nigeria. Different concentrations of 1.0, 2.5, and 5% w/v of the bio-oils were used to treat 9 cm Whatman filter paper placed in a separate Petri dish (10 cm). Ten seeds per treatment were placed in each Petri dish and were covered, and then placed inside a dark cupboard. Controls were similarly prepared with distilled water. The tests were carried out with three replicates per treatment and all the Petri dishes were kept in a dark cupboard at room temperature and relative humidity. The experiments were carried out for 7 days and 14 days after the introduction of the seed. The percentages of seedling growth inhibition were calculated by difference between controls and treated samples using Equation 2.

$$\% \text{ inhibition} = \frac{A-B}{A} \times 100 \quad (2)$$

where A is the average length of shoot and root in control, and B is the average length of the shoot/root in the tested treatments.

The results of the experiments were expressed as the mean \pm standard deviation (SD). The statistical analysis to test the significance differences were conducted using ANOVA and Duncan test, with a $p < 0.05$, which indicates significance. Statistical Package for Social Science (SPSS) version 26 was used for statistical treatment of the data.

3 Results and discussion

3.1 Physicochemical characteristics

Bio-oils were successfully prepared from empty palm fruit bunches at different pyrolytic temperatures. The percentage yields of the bio-oils are 22.07 ± 0.21 , 35.13 ± 1.89 and 37.47 ± 1.82 at 400 °C, 500 °C, and 600 °C, respectively. The results obtained for the proximate,

specific gravity and density of the bio-oil are presented in Table 1. The results showed that the bio-oils had pH values in the range of 2.29 and 2.57. This phenomenon indicates that the three bio-oils are acidic and the high acidity of the bio-oils means that they are corrosive. A number of acidic compounds, such as oleic acid, benzoic acid, and carbamic acid, are present in bio-oils [5]. The moisture contents of the bio-oils are in range of 10.56 and 16.32% wt. These values are higher than those of petroleum diesel and heavy fuel oils, which have low moisture contents of about 0.1% wt. This observation could be due to the high water content of the feedstock, as a result of this, a large amount of water was released into the bio-oils during pyrolysis (lignocellulosic) [25]. The densities of the bio-oils range between 1.0 and 0.92 g/cm³, an indication that the bio-oils are denser than light petroleum oil (diesel oil) with densities of 0.85 g/cm³. The density of heavy oil (0.96) is closer to those of the bio-oils. The specific gravities (0.92 – 1.0) are closer to the values (1.1 – 1.2) reported in literature [26].

The FTIR data and the peak assignments of the empty palm fruit bunches bio-oils at 400 °C and 600 °C are presented in Table 2. The major functional groups present in the bio-oils are O–H, C≡C, C=O, C–O, C–O–C and C–X (X = halide). The results presented in Table 2 are similar to what had been previously reported [27]. According to Keiluweit et al. [28], the C–C stretching vibration at 1595 cm^{–1} could be related to the presence of aliphatic carbon groups or deformation of aromatic groups C=C.

The GC-MS analyses of the bio-oils at 400 °C and 600 °C, with heating rate of 15 °C/min, are presented in Tables 3 and 4, respectively. The standard NIST MS (The National Institute of Standard and Technology Mass Spectral) Library of the bio-oils were used to identified compounds in the tables. The percentage peak areas of the identified compounds were recorded in relation to the yield of the products. There are similarities in the chemical composition of the bio-oils. Most of the common compounds are phenols and phenol derivatives, alkanes, and acids. Mullen et al. [29] reported similar chemical compounds for bio-oils.

Table 1 Physicochemical analysis of the empty palm fruit bunches bio-oils

Temperature (°C)	pH	Specific gravity	Density (g/cm ³)	Moisture content (% wt)	Ash content (% wt)
400	2.29 ^b \pm 0.01	0.93 ^a \pm 0.04	1.00 ^a \pm 0.10	10.56 ^c \pm 0.51	0.31 ^a \pm 0.01
500	2.36 ^b \pm 0.02	1.10 ^a \pm 0.10	0.98 ^a \pm 0.01	14.23 ^b \pm 0.21	0.42 ^a \pm 0.02
600	2.57 ^{ab} \pm 0.02	1.02 ^a \pm 0.01	0.92 ^a \pm 0.02	16.32 ^a \pm 0.45	0.52 ^a \pm 0.01

3.2 Percentage seed germination

The results obtained for percentage seed germination are presented in Table 5. The results obtained showed that different concentrations of empty palm fruit bunches bio-oils significantly reduced the germination of Tomatoes,

Okra and Amaranthus seeds. For 5.0% (w/v) concentration of empty palm fruit bunches bio-oil, Amaranthus had the highest percentage seed germination of 26.67%, tomatoes and okra had no percentage seed germination for bio-oil at 400 °C. Percentage seed germination for Okra and

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Amaranthus was 10.00% each, while Tomatoes had no percentage germination for the bio-oil at 500 °C. Okra had the highest percentage germination (13.33%), followed by Amaranthus (10.00%) for bio-oil at 600 °C. For 2.5% (w/v) concentration empty palm fruit bunches bio-oils at different temperatures, the percentage seed germinations followed this order: Amaranthus (50.00%), Okra (40.00%) and Tomatoes (3.33%); Okra (30.00%), Amaranthus (20.00%) and Tomatoes 0.00%; and Amaranthus (26.67%), Okra (23.33%) and Tomatoes (3.33%) for empty palm fruit bunch bio-oil at 400 °C, 500 °C and 600 °C,

respectively. For 1.0% (w/v) concentration of empty palm fruit bunches bio-oils at different temperatures, the percentage seed germinations are in the order: Amaranthus (56.67%), Okra (43.33%) and Tomatoes (6.67%); Amaranthus (60.00%), Okra (50.00%) and Tomatoes (10.00%); and Amaranthus (40.00%), Okra (53.33%) and Tomatoes (10.00%) for empty palm fruit bunches bio-oil at 400 °C, 500 °C and 600 °C, respectively. It can be deduced from the study that the percentage of the seed germination decreases as the concentration of the bio-oil increases.

Table 2 FTIR analysis of the empty palm fruit bunches bio-oils

Temperature (°C)	Wavenumber (cm ⁻¹)	Peak Assignment
400	3331.95	O–H stretching (phenol, alcohol, water, Keiluwiet <i>et al.</i> [28])
600	3329.16	
400	2923.84	C–H stretching
600	2925.25	
400	2093.82	C≡C stretching
600	2153.58	
400	1704.19	C=O stretching
600	1698.01	
400	1595.00	C–C stretching
600	1595.00	
400	1463.19	CH ₃ deformation
600	1462.16	
400	1373.42	O–H bending of phenolic (Keiluwiet <i>et al.</i> [28])
600	1374.93	
400	1227.01	C–O–C stretching aryl-alkyl ether linkage
600	1232.47	
400	1110.56	Symmetric C–O stretching (Keiluwiet <i>et al.</i> [28])
600	1109.13	
400	752.76	C–X, Alkyl halide
600	752.67	

Table 3 Compounds in empty palm fruit bunches bio-oil at 400 °C

S/NO	Identified Compound	Retention Time (min)	Peak Area (%)	Molecular Formula
1	Pyridine	4.34	0.12	C ₇ H ₆ N ₂
2	2-Furanmethanol	7.94	0.13	C ₅ H ₆ O ₂
3	Mequinol	17.05	4.47	C ₇ H ₈ O ₂
4	<i>p</i> -Cresol	17.91	2.18	CH ₃ C ₆ H ₄ OH
5	Phenol	28.83	10.95	C ₆ H ₅ OH
6	Vanillin	30.57	0.29	C ₈ H ₈ O ₃
7	3,5-Dimethoxy-4-hydroxytoluene	32.14	1.37	C ₉ H ₁₂ O ₃
8	Butyrovannillone	37.55	0.17	C ₁₁ H ₁₄ O ₃
9	2,6-dimethoxy-4-(2-propenyl)-Phenol	37.91	0.18	C ₁₁ H ₁₄ O ₃
10	Benzaldehyde		0.22	C ₇ H ₆ O
11	Ethanone	40.24	0.38	C ₂ H ₃ O
12	<i>n</i> -Hexadecanoic acid	42.81	0.48	CH ₃ (CH ₂) ₁₄ COOH
13	9-Octadecenoic acid	50.63	0.11	C ₁₈ H ₃₄ O ₂
14	Oleic acid	54.17	0.13	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH

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Table 4 Compounds in empty palm fruit bunches bio-oil at 600 °C

S/NO	Identified Compound	Retention Time (min)	Peak Area (%)	Molecular Formula
1	Phenol	16.79	7.33	C ₆ H ₅ OH
2	<i>p</i> -Cresol	17.67	3.25	CH ₃ C ₆ H ₄ OH
3	2,6-dimethoxy-phenol	28.60	7.71	C ₈ H ₁₀ O ₃
4	Benzoic acid	28.90	0.30	C ₇ H ₆ O ₂
5	Vanillin	30.53	0.44	C ₈ H ₈ O ₃
6	3,5-Dimethoxy-4-hydroxytoluene	32.12	1.37	C ₉ H ₁₂ O ₃
7	Butyrovannillone	37.63	0.44	C ₁₁ H ₁₄ O ₃
8	Heptacosane	63.35	0.28	C ₂₇ H ₅₆
9	1,1'-Biphenyl	67.47	0.02	C ₆ H ₅ C ₆ H ₅

These results agreed with those obtained by Sikolia and Ayuma [22] and Anwar et al. [30]. In the report of Sikolia and Ayuma, the germination percentage of cowpea seeds decreased with an increase in the concentration of shoot aqueous extract of *Eucalyptus saligna*. Similar results were reported by Ilori et al. [31] and Malik [32]; these authors reported that the solvent extract reduced germination percentages and germination rates. Similarly, Zribi et al. [33] reported that the percentage seed germination of

Lactuca sativa in the presence of aqueous extract at different concentrations of Tunisian and Indian varieties of *Nigella sativa* seeds decreased with an increase in concentration of the extract. However, there were no significant differences ($p < 0.05$) in germination percentages among the bio-oils (individual temperatures) for Tomatoes seed, but there were significant differences among bio-oils (individual temperatures) for Amaranthus and Okra seeds as shown in Table 5.

Table 5 Percentage seed germination of empty palm fruit bunches bio-oil on three selected seeds

Temperature (°C)	Concentration (%)	Crops		
		Tomatoes	Okra	Amaranthus
400	1.0	6.67±5.77 ^b	43.33±20.82 ^b	56.67±15.28 ^{ab}
	2.5	3.33±5.77 ^b	40.00±20.82 ^b	50.00±20.00 ^{bc}
	5.0	0.00±0.00 ^b	0.00±0.00 ^c	26.67±15.28 ^c
	Control	90.00±10.00 ^a	96.67±5.77 ^a	96.33±5.77 ^a
500	1.0	10.00±10.00 ^b	50.00±26.46 ^b	60.00±17.32 ^a
	2.5	0.00±0.00 ^b	30.00±10.00 ^{bc}	20.00±10.00 ^b
	5.0	0.00±0.00 ^b	10.00±10.00 ^c	10.00±10.00 ^b
	Control	95.00±10.00 ^a	93.33±5.77 ^a	98.33±15.28 ^a
600	1.0	10.00±10.00 ^b	53.33±5.77 ^b	40.00±20.00 ^b
	2.5	3.33±5.77 ^b	23.33±5.77 ^c	26.67±5.77 ^c
	5.0	0.00±0.00 ^c	13.33±5.77 ^c	10.00±10.00 ^c
	Control	89.00±10.00 ^a	94.33±5.77 ^a	100.33±5.77 ^a

Values are means of triplicate ± standard deviation. Means of the column followed by the same superscript letters are not significantly different at $p < 0.05$.

3.3 Seedling growth bioassay

The results of the percentage inhibition of the seedling growth are presented in Table 7. From the results obtained, it was observed that the percentage inhibitions of seedling growth of shoot and root length of Tomatoes, Okra and Amaranthus significantly increased as the concentration of empty palm fruit bunches bio-oils increased. For 5.0% (w/v) concentration of the bio-oil, the inhibition activities were high for shoot length and root length at 7th and 14th days for Tomatoes, Okra and Amaranthus seeds at all temperatures. For 2.5% (w/v) concentration of the bio-oil, the inhibition activities were also high for shoot length at 7th and 14th days for Tomatoes, Okra, and Amaranthus at all temperatures. For 1.0% (w/v) concentration of the bio-

oil, the inhibition activities were substantially good for root length at 7th day for tomatoes (all temperatures); bio-oil at 400 °C for Okra, bio-oils at 400 °C and 500 °C for Amaranthus, bio-oils at 500 °C and 600 °C for Okra. At 600 °C. Amaranthus has highest value for shoot length at 7th day. Similarly, the inhibition activities were high for shoot length at 14th day for okra at all temperatures for Tomatoes at 500 °C, but high for root length at day 14th for Amaranthus and bio-oils at 400 °C, 600 °C for Tomatoes. The result in Table 7A-C showed that the seedling growth on percentage inhibition of shoot and root length increased as the bio-oil concentration was increased. The result from the present study is in agreement with the reports of Cheema and Khaliq [38], Rafiqul-Hoque et al. [39], and Adetayo et al. [40]. These authors emphasized

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that the inhibitory effects were proportional to the concentration of the extracts, that is, the higher concentration of inhibiting species, the stronger the inhibition. In some cases, low concentrations of inhibiting substance showed stimulatory effect. Sarmin [41] observed 14% and 8% reduction in seed germination of cowpeas and

soya bean treated with the water extract of *C. odorata* leaves and stems, respectively. For the shoot and root lengths of Tomatoes, Okra, and Amaranthus seedlings treated with empty palm fruit bunches bio-oils at different temperatures were observed to be significantly different compared to the control.

Table 7A Percentage inhibition of empty palm fruit bunches bio-oil against Tomatoes

Temperature (°C)	Concentration (%)	Tomatoes			
		7 days		14 days	
		Shoot	Root	Shoot	Root
400	1.0	42.11±21.54 ^b	53.33±10.10 ^b	57.14±14.29 ^b	69.70±8.02 ^b
	2.5	83.33±8.49 ^a	73.24±22.57 ^b	91.23±0.00 ^a	86.27±5.89 ^a
	5.0	97.67±2.33 ^a	97.50±2.50 ^a	98.33±1.44 ^a	97.14±2.85 ^a
	Control	16.67±5.77 ^c	16.67±5.77 ^c	10.00±5.77 ^c	8.23±5.77 ^c
500	1.0	52.63±20.89 ^b	53.33±15.07 ^c	77.14±12.45 ^b	76.34±8.12 ^b
	2.5	90.12±1.07 ^a	72.98±4.68 ^b	94.92±2.93 ^a	91.16±5.14 ^a
	5.0	99.22±1.35 ^a	98.33±2.89 ^a	99.26±1.28 ^a	98.10±3.30 ^a
	Control	6.67±4.45 ^c	6.67±4.45 ^d	8.92±2.14 ^c	3.98±1.56 ^c
600	1.0	42.10±30.35 ^b	54.17±21.55 ^b	61.76±5.89 ^b	65.52±9.12 ^b
	2.5	83.95±7.48 ^a	72.97±16.88 ^b	91.23±5.27 ^a	91.51±2.99 ^a
	5.0	99.22±1.35 ^a	98.33±2.89 ^a	99.19±1.41 ^a	98.99±1.75 ^a
	Control	10.76±3.77 ^b	9.34±4.19 ^c	11.01±1.34 ^c	8.17±3.78 ^c

Values are means of triplicates ± standard deviation. Means of the column followed by the same superscript letters are not significantly different at $p < 0.05$

Table 7B Percentage inhibition of the bio-oils against Okra

Temperature (°C)	Concentration (%)	Okra			
		7 days		14 days	
		Shoot	Root	Shoot	Root
400	1.0	24.24±29.23 ^b	37.63±6.71 ^c	55.21±15.42 ^b	52.78±13.39 ^b
	2.5	69.93±12.76 ^a	65.79±11.47 ^b	83.33±7.22 ^a	77.09±6.51 ^a
	5.0	90.38±8.38 ^a	84.85±4.73 ^a	86.21±12.43 ^a	83.33±13.89 ^a
	Control	6.67±5.77 ^b	6.67±5.77 ^d	16.67±5.77 ^c	10.54±5.77 ^c
500	1.0	62.57±9.01 ^b	46.67±24.02 ^b	42.71±13.01 ^b	38.89±30.71 ^b
	2.5	79.10±4.26 ^b	67.36±8.42 ^b	85.00±10.00 ^a	79.17±10.05 ^a
	5.0	98.75±2.17 ^a	96.79±5.55 ^a	96.88±5.41 ^a	90.91±15.74 ^a
	Control	3.33±3.32 ^d	6.67±5.77 ^c	5.11±1.02 ^c	2.56±2.51 ^c
600	1.0	63.33±8.12 ^b	58.33±10.10 ^b	58.34±12.63 ^b	47.22±26.78 ^b
	2.5	78.70±17.86 ^a	62.96±16.14 ^b	88.33±8.78 ^a	83.34±11.83 ^a
	5.0	95.11±5.05 ^a	93.83±4.28 ^a	94.25±9.95 ^a	93.94±10.50 ^a
	Control	10.00±0.00 ^c	10.00±0.00 ^c	7.34±0.61 ^c	5.83±1.23 ^c

Values are means of triplicates ± standard deviation. Means of the column followed by the same superscript letters are not significantly different at $p < 0.05$

Table 7C Percentage inhibition of the bio-oils against Amaranthus

Temperature (°C)	Concentration (%)	Amaranthus			
		7 days		14 days	
		Shoot	Root	Shoot	Root
400	1.0	38.64±17.75 ^c	48.89±18.36 ^b	63.96±3.12 ^c	64.51±5.59 ^b
	2.5	50.00±11.47 ^b	40.69±15.45 ^{bc}	78.84±3.33 ^b	71.67±2.89 ^b
	5.0	98.41±2.75 ^a	97.78±3.61 ^a	97.78±1.92 ^a	96.58±2.89 ^a
	Control	8.56±6.00 ^d	5.78±3.87 ^d	13.33±5.77 ^d	13.33±5.77 ^c
500	1.0	34.96±18.63 ^c	41.88±22.11 ^b	51.43±11.15 ^c	55.24±13.31 ^b
	2.5	61.77±5.09 ^b	29.63±19.51 ^b	74.79±11.26 ^b	76.85±3.21 ^a
	5.0	89.52±3.30 ^a	78.67±14.05 ^a	97.85±0.94 ^a	91.43±4.95 ^a

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600	Control	3.83±8.30 ^d	6.12±10.00 ^d	9.19±0.12 ^d	8.87±2.11 ^c
	1.0	46.34±15.99 ^b	33.33±14.43 ^c	50.00±19.24 ^b	55.56±15.39 ^b
	2.5	79.41±20.38 ^a	68.52±11.56 ^b	95.43±1.13 ^a	88.57±4.95 ^a
	5.0	95.50±3.12 ^a	91.67±0.00 ^a	98.41±0.00 ^a	97.06±0.00 ^a
	Control	10.00±5.77 ^c	4.87±2.81 ^d	6.23±5.77 ^c	5.29±2.91 ^c

Values are means of triplicates ± standard deviation. Means of the column followed by the same superscript letters are not significantly different at $p < 0.05$.

4 Conclusions

Bio-oils were successfully obtained from bunches of empty palm fruit. Inhibitory effects of the bio-oils against seed germination and seedling growth of Tomatoes, Okra and Amaranthus were investigated. Bio-oils obtained from empty palm fruit bunches at pyrolytic temperatures of 400 °C, 500 °C and 600 °C exhibited allelopathic properties on the selected seeds. The bio-oils reduced the percentage seed germination and seedling growth. The inhibitory activities of the bio-oils on shoot and root of the plants were dependent on the concentration of the bio-oils. The bio-oils obtained from the lignocellulosic waste (bunches of empty palm fruit) could be used agrochemicals.

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INTERDISCIPLINARITY IN SCIENTIFIC RESEARCH: THE EMPLOYMENT OF TECHNOLOGICAL TOOLS IN DATA ANALYSIS WITHIN AN INTERDISCIPLINARY CONTEXT

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Abstract: This article aims to present the importance of using technologies in data analysis for scientific research in an interdisciplinary context. It is known that technology currently constitutes a tool in common between different areas of knowledge, which makes it an instrument of great relevance for both parties, as it facilitates the researcher to analyze large amounts of information in different areas in a technical, practical and technical way, minimizing the margin of error. These relationships between different areas of knowledge, especially within scientific research, are very important to have a better understanding of a subject under discussion, and to find explanations for such facts and questions. In view of this, to evidence such facts, bibliographical research was carried out as methodological procedures, in which already published works were used as a source, such as: articles, master's dissertation, doctoral thesis and books, to validate/relate in order to present the contributions of several authors on the proposed theme. Finally, the research findings could show that, with the advancement of technology, as discussed in the text, much software with the help of the internet and some electronic devices such as notebooks, tablets, smartphones, among other resources, have been favoring this type of work and providing the speed and precision in obtaining and analyzing data, improving the execution of tasks in an agile, precise and compensatory way.

1 Introduction

Interdisciplinarity is nowadays a widely discussed science topic. It deals with a diverse body of disciplines and areas of knowledge, which interact in a way to arrive at new perspectives on a common object of investigation, uniting knowledge and avoiding information fragmentation. The relationship between different areas of knowledge, especially within scientific research, is extremely important in order to better understand a subject under discussion, as well as to find explanations for facts and questions [1]. In addition, there are common tools between the areas, which are used to process this information. Among them, technological tools deserve special attention since they are highly useful in collecting information, and storing and manipulating data [2].

Currently, we live in a world full of technological tools that help us in our various daily activities. The use of these tools has been widely recommended because they facilitate the execution of tasks, thus helping to achieve goals in a simplified and less laborious way. In addition, they are used in many areas of knowledge, in its modalities and specific applications, whether through

software tools or electronic devices, etc., helping them in their actions and achieving results [3].

Under this consideration, the main point of reflection presented in this article is the use of technological tools in the process of data analysis within an interdisciplinary context. In order to achieve this, we used bibliographic sources such as articles, master's, doctoral thesis and books, seeking to validate and relate technological tools with data analysis and interdisciplinarity.

2 State of the Art

2.1 Technological tools

We currently live in an era of technological development never seen before, in which mankind, through our needs and ability to reinvent ourselves in the face of challenging situations, have developed and improved technological knowledge that benefits us in the most different areas and fields of action, becoming an indispensable instrument in our lives. Such technological knowledge contributed to the creation of tools, which provided solutions and improvements in several sectors (such as: health, economy, education, etc.), since they help

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in different activities, fulfilling purposes and at the same time serving as basis for the creation of new ideas, or improvements, from the perspective of other researchers [3].

Furthermore, the emergence of electronic devices and the arrival of the internet, besides revolutionizing the media, gave us the ability to carry out different works, including creating, analyzing and simulating tasks in a virtual way, which gave the users the ability to communicate among each other, as well as sharing and accessing information in real time. Some devices even have a compact design that fits in the palm of the hand (Figure 1), which can be easily transported inside a pocket [4].



Figure 1 The smartphone as a world of information in the palm of the hand.

Nowadays, with the help of technological/digital tools, it has become possible to verify whether certain ideas, theories, hypotheses, etc., can succeed or not when put into practice, as well as identify possible failures in case of failure. This type of action can be planned and tested through applications in scenarios involving computer simulations, in which in these spaces it is possible to simulate possible situations based on real trajectories/activity, in which from this it is possible to have a futuristic view of such facts or analyzed data, as well as extracting information that can serve as a basis for further research and generation of results [5]. The use of these tools are extremely important in the decision-making process, especially when the issues dealt with may in the future cause damage to people's health and lives, preventing them from possible eventualities that may happen by taking hasty actions [6].

2.2 Data analysis

According to Mozzato and Rzybovski [7], data analysis consists in one of the most important stages of a research project, since it allows the researcher to make decisions strategically, extract information of interest and reach conclusions on the subject of interest. This activity constitutes of a systematic in-depth study of data regarding an area of interest. It represents a high level of

significant work, which in many cases can become exhausting activities, depending on the method, tool, or field of action chosen to be analyzed.

Data analysis, according to Bardin [8], can be defined as:

A collection of communication analysis tools that seek to obtain, through systematic methods and content description objectives indicators (quantitative or not) that allow the inference of knowledge regarding the conditions of production or reception of such messages [8].

Bortoloti and Bruns [9], emphasizes the importance of attention in the data analysis process, because when performing this type of action in a hasty or erroneous way, it can compromise all the carried-out research, producing false conclusions and putting to waste years of research in a chosen field of study. Minayo [10] states that:

We should describe with clarity how data is organized and analyzed. For instance, content analyses, of speech, or dialectic analysis are possible proceedings for analysis and interpretation of data and each one of these modalities advocates a different treatment for the organization and systematization of data [10].

Today, thanks to technology development, it is possible to analyze large amounts of data in different areas through technical, fast and practical ways, with the help of cutting-edge technological tools, predefined to work according to the situations of interest. Currently there are platforms capable of automating the capture of information and performing interpretations intelligently, applying Artificial Intelligence (AI), which consists of identifying patterns of behavior about the information being observed and from there extracting data from different perspectives and reach conclusions in a systematic way [11].

2.3 Interdisciplinary context

According to Cesco [12], interdisciplinarity consists on the relationship between different disciplines or areas of knowledge, in order to interact and seek a better understanding of a given problem. The objective of this interaction is to treat common research objects from different professional perspectives and their related areas, in order to seek answers to complex problems (Figure 2), which a single discipline or specific area of knowledge is not capable of solving. This relationship tends to provide an exchange of individual knowledge, which, when interconnected, lead the achievement of better results on the issue under discussion.

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Figure 2 Schematic representation of interdisciplinary work and the contributions that the interaction between disciplines can offer in search for problem solution.

In order to work in an interdisciplinary way, it is necessary to have a common object of study between different disciplines, and based on it, one might raise a contextualized approach on the subject addressed. Bicudo [13] in his article entitled “Interdisciplinary research: a possibility for the construction of scientific/academic work”, questions how to work in an interdisciplinary way. According to him:

It is always necessary to have a theme as a guideline for the investigation. A sufficiently comprehensive subject, which approaches do not fit within the limits of a discipline, pushing its limits and not adapting to its methods. However, the inherent rigor of scientific procedures must be observed, so that researchers do not inadvertently appropriate it, without a cautious study carried out with the support of researchers from interconnected disciplines, the research and respective results or discussions exposed outside these disciplines. It requires that you work in a group, respecting each other, that you treat knowledge as activity and not as merchandise, that you have the humility to listen to each other and express questions and naive doubts [13].

Still in this scenario, when it comes to the common object of study, there are different ways of analyzing this data that will serve as basis for future contextualization. Among them, we highlight the use of technological tools to achieve this specific purpose, through intelligent software, electronic forms, etc., which can help in the process of collecting data and interpreting information systematically [2].

3 Methodology

This article consisted of bibliographical research, in which we used already published works, in order to

present the contributions of several authors on the proposed subject. In Gil's view [14], “bibliographic research is developed based on material already prepared, consisting mainly of books and scientific articles”. Pereira and Pacheco [15] say that this kind of research is used in almost all sorts of study, however, there are certain cases in which studies are developed specifically based on bibliographic research.

Thus, the research carried out here was qualitative, which emphasizes the inductive interpretation as the main characteristic, despite the existence of other investigation methods. According to Minayo [10], “it answers very particular questions. It is concerned, in the social sciences, with a level of reality that cannot or should not be quantified”.

4 Conclusion

The present study made it possible to reflect on the applicability of technological tools in an interdisciplinary context, emphasizing their importance in analyzing and obtaining data.

In this context, it was observed based on the bibliographic research of authors, that scientific research is not based only on a content, theme or topic, it involves several aspects, that is, we develop the studies taking into account various disciplines, tools and equipment, analysis multiplies, several lines of science to arrive at the result. And through such interactions, it is necessary to use tools that provide researchers with improvements in the execution of these tasks, in an agile, precise and compensatory way.

In view of this, the research findings could show that, with the advancement of technology, as discussed in the text, many software with internet aid and some electronic devices such as notebooks, tablets, smartphones, among other resources, have been favoring this type of work and providing the speed and accuracy in obtaining and analyzing data. Still from this perspective, it is expected that in the future, works where other researchers will be concerned about adopting such tools in their research scenarios, so that they can improve their results and their assertiveness.

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INTERDISCIPLINARITY IN SCIENTIFIC RESEARCH: THE EMPLOYMENT OF TECHNOLOGICAL TOOLS IN DATA ANALYSIS WITHIN AN INTERDISCIPLINARY CONTEXT

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HAVE COMPANIES IN INDONESIA DISCLOSE ENVIRONMENTAL ACCOUNTING?

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diocaesar@stiesam.ac.id (corresponding authors)**Keywords:** Fekrat scoring, environmental, CSR, business continuity, Indonesia.

Abstract: Environmental accounting is an effort to minimize the negative impact on the environment of the company's operational activities, both monetary and non-monetary. This effort is carried out by identifying the environmental impacts of the company's operations and the costs of handling these impacts. There have been several regulations referring environmental accounting disclosures, but the application of these disclosures is still voluntary. The investigation focuses on the importance of environmental accounting disclosures, i.e. to find out whether companies in Indonesia have disclosed environmental problems from their operations. The data is supported by the annual reports of companies listed on the Indonesia Stock Exchange (IDX) in 2021 in all industries. Methods of data analysis using scoring techniques or assessments using the Frictional scoring method and descriptive analysis. We found that the average disclosure in each aspect of the assessment model Fekrat shows zero and one. The other side shows that most of the companies sampled in this study have not made environmental disclosures. The contribution of this finding can serve as a basis for policymakers in Indonesia to assess the effectiveness of related regulations in environmental protection efforts. Apart from that, other facts also show the need for the legitimacy of the IDX as a capital market operator to require companies to disclose environmental accounting in their annual reports. The professional practical and research implications for the future relative also to the concept of dealing with, changing, and significantly implementing consortia in sustainable environmental accounting mechanism.

1 Introduction

The main purpose of accounting is to provide information related to business continuity for company stakeholders, both management and capital owners (investors and creditors). The information is summarized in the financial statements consisting of income statements, changes in equity, financial position, and cash flow, and accompanying notes [1].

The information in these reports is quantitative and focuses on information about the company's ability to survive and make a profit. Meanwhile, in carrying out its operations, there are both social and environmental impacts that must be disclosed by the company. Therefore, the accounting must be able to answer the needs of information regarding the impact of these, one of which related to the socio-ecological impact resulting from business activities [2].

Environmental accounting is an effort to minimize the negative impact on the environment from the company's operational activities both monetary and non-monetary. Environmental accounting is also related to the sustainability report. Sustainability reports are reports produced by business organizations to disclose related environmental, social, and good governance performance [3]. The publication of sustainability reports uses the Global Reporting Initiative (GRI) standard established by the United Nations Environment Program (UNEP), the Coalition for Environmentally Responsible Economies (CERES), and the Tellus Institute in 2000. GRI is a standard in the practice of publicly reporting organizations related to the impact of economic, social, and environmental. The existence of GRI aims at greater organizational transparency and accountability.

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In Indonesia, the government has issued Government Regulation (PP) number 47 of 2012 concerning the "Implementation of Corporate Social and Environmental Responsibility" in April 2020. With this regulation, the obligations of business organizations have serious consequences on the recognition, measurement, recording, presentation, and disclosure of accounting related to environmental aspects [4]. Another rule is Regulation of the Financial Services Authority (OJK) No. 51/POJK.03/2017 concerning the "Implementation of Sustainable Finance for Financial Services Institutions, Issuers, and Public Companies" [5]. Even though there are rules, basically sustainability reports are voluntary. Based on the OJK report in 2021, there is almost nine percent of companies listed on the Indonesia Stock Exchange (IDX) have published sustainability reports.

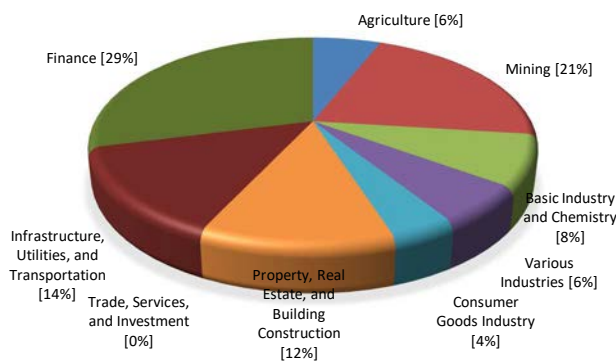


Figure 1 Percentage of sustainability report publishers by sector. Source: [6]

Figure 1 summary the finance sector has the highest percentage of other industries, namely by 29%. The high level of issuance of sustainability reports in the financial sector is a form of compliance from financial institutions that are directly overseen by the OJK based on the regulations and guidelines for the sustainability report from OJK. However, the high issuance of sustainable reporting (SR) in the financial sector does not directly indicate the high disclosure of environmental issues, but only shows social responsibility. The next ranking is in the mining sector whose operations directly utilize natural resources. This is in line with PP number 47 of 2012 which requires every business activity that directly utilizes natural resources to disclose environmental accounting. Besides, in 2016 four mining companies made sustainability reports with more than 50% disclosure under GRI guidelines [7].

However, when compared with several countries in the world, such as countries in America, Europe, and Asia-Pacific, until 2019 Indonesia still occupies a position with a low average percentage of SR disclosure [8]. SR disclosure in Indonesia is still at 9% above Pakistan, Poland, and Russia. The countries with the highest disclosure rates are Japan, America, the United Kingdom, and China. The sector with the highest average SR disclosure is in the energy and utility sector which is a

business that directly utilizes natural resources. Furthermore, Singer [8] also explain two main factors that encourage the disclosure of SR are the presence of pressure from stakeholders and the existence of regulations that require SR accounting. This is in line with the results of research by Liu [9] which shows that the steel industry in China has problems in disclosing environmental accounting because there are no applicable environmental accounting standards, strict rules, and supervision of the company. Likewise, according to Monteiro & Guzmán [10] during 2002-2004 environmental accounting disclosures in companies in Portugal increased after the existence of new accounting rules and standards.

Based on the fact that environmental accounting disclosure is still very low in Indonesia and the existence of regulations related to environmental accounting in Indonesia, this study aims to identify the extent to which companies in Indonesia disclose environmental issues from their operations. This research is a continuation of Fadillah & Djaddang [11] research which examines the disclosure of environmental accounting in ASEAN countries. Research on environmental accounting disclosures has been done a lot, but no one has analyzed the substance of the disclosure made by the company [12].

In this study, we trying to assess the extent of environmental disclosure aspects in sustainable reports on Indonesian industries. The assessment is based on the average disclosure of environmental aspects with a scoring model from Fekrat. This study involved eight industrial sectors listed on the Indonesia Stock Exchange (IDX). To simplify the research results table, each industry is labeled such as consumer goods, agriculture, mining, various industries, property, real estate, and building construction, basic and chemical, infrastructure, utilities, and transportation, and trade, services, and investment.

The value of the originality of the study is an important part of an evaluation for further researchers. Weaknesses must be highlighted against companies that have only prioritized business aspects so that they must also understand problems that are relevant to the environment and social issues. That way, business prospects can have a systematic impact through deferred burdens on companies willing to spend financially on environmental and social aspects.

The study's contribution focused on two elements covering practical and academic. Practically, companies that are members of IDX need operational standards that highlight reports related to environmental accounting. This intended so that policies that are oriented towards environmental improvement (such as CSR) channeled through a competently planned road map. On the academic side, the output of this study serves as a conceptual reference for exploring environmental effects caused by company activities. At least, they can reduce this to a clear standard in order to minimize risk.

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1.1 The urgency of CSR

Now is the era of the open society that increasingly pays attention to the environment, both social and environmental. Associated with these things appear the term Corporate Social Responsibility (CSR), which develops and began fame in 1998, from the book by John Elkington [13]. The book discusses three important elements to achieve sustainable development, i.e. economic growth, environmental protection, and social equity, or better known as profit, planet, and people [14]. With the development of CSR and the inclusion of a green company era, companies must incorporate elements of environmental impact into their financial statements [15]. The report is part of a form of CSR implementation, commonly called environmental accounting. Environmental accounting is born because environmental problems develop rapidly, from pollution, forest preservation, population growth, to the depletion of natural resources. Also, environmental problems arise related to energy supply, biodiversity, species extinction, climate change, and other things [16].

The emergence of ideas about CSR and its elements is related to the theory of legitimacy. According to the theory, there needs to be an adjustment between the operational activities of business organizations with the values held by the community, one of which is not disturbing environmental aspects that can harm people's lives [17]. When there is no match between these two things, the position of the two parties can be threatened. An example of the case is the Lapindo mudflow in 2006 caused a large impact on several aspects of community life, such as the existence of a pool with a height of up to six meters in the settlement, victims affected more than 8,200 people, houses damaged approximately 1,683 units, agriculture and plantation areas were damaged approximately 200 ha, more than fifteen factories stopped production activities and laid off more than 1,873 people, damaged educational facilities, damaged infrastructure facilities and infrastructure in the form of electricity and telephone networks, and obstruction of the Malang-Surabaya toll road that affects production activities in the Ngoro (Mojokerto) and Pasuruan areas [18]. As the company responsible for the Lapindo mud case, Lapindo Brantas Inc. must have incurred a loss of IDR 3.8 trillion to compensate for the affected people's land.

1.2 Environmental accounting

Environmental accounting is very important as a form of transparency and accountability to the stakeholders of each company's activities to prevent, reduce, and stop environmental impacts that are happening or that might potentially occur in the future [17]. Environmental accounting can be interpreted as incorporating elements of

environmental costs into accounting practices, both for corporate and government financial statements. Environmental accounting is an effort to prevent, reduce, and avoid environmental impacts, starting from the re-improvement of events that cause disasters for all aspects of these activities [19].

Associated with the low SR disclosure which also shows the low level of environmental accounting disclosure in addition to being caused by external factors (stakeholder and government pressure) is also caused by internal company factors [20]. One of them is the lack of knowledge and ability of company management related to the disclosure of non-financial information, ranging from identification, measurement, to the presentation and disclosure of environmental information [11]. Also, management's fear of poor publicity and perceived poor performance also contributed to the low environmental accounting disclosure.

In some countries such as Pakistan, environmental disclosure is significantly related to environmental performance in companies in Pakistan. Companies with good environmental performance and low emission levels disclose more environmental information [16]. Besides, companies in Nigeria have a positive relationship between environmental accounting and the quality of accounting disclosure for shipping companies in Nigeria [21].

2 Method and measurements**2.1 Data set**

The population in this study are all companies in all industries listed on the IDX. Samples were selected using purposive sampling and the criteria are company is listed on the Stock Exchange with an IPO before 2022, a published complete annual report ended on December 31 of 2021, and disclosed environmental accounting during 2021 in Indonesia. It applied specifically the technique to industrial companies which are selected based on monetary aspects (financial, assets, and profits) and non-monetary aspects (industrial waste, environment, social, and local culture). These two aspects interrelated, where from the monetary dimension it focuses on companies that get maximum profit and the non-monetary dimension includes the urgency of controlling CSR on the impact of environmental pollution felt by residents which has the potential to cause conflict.

In this study, the finance industry does not fit into the criteria of the sample because no company disclosed environmental accounting in its sustainability report [23,24]. So, we exclude the finance industry from the sample. From *Table 1*, the data collection is done by downloading the annual report published by the IDX through the official website www.idx.co.id and the company's official website.

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Table 1 Distribution of companies by sector

Sector	Population		Sample		% of samples
	Total	(%)	Total	(%)	
Consumer Goods Industry	57	8.4	37	12.1	64.9
Agriculture	22	3.2	16	5.6	77.3
Mining	49	7.2	34	11.2	69.4
Various Industries	50	7.4	32	10.5	64.0
Property, Real Estate, and Building Construction	88	13.0	47	15.4	53.4
Finance	92	13.5	0	0	0
Basic Industry and Chemistry	73	10.8	51	16.7	69.9
Infrastructure, Utilities, and Transportation	79	11.6	32	10.5	40.5
Trade, Services, and Investment	169	24.9	55	18	32.5
Total	679	100.0	305	100.0	44.9

Source: [22]

2.2 Analysis data

Data analysis in this study uses descriptive analysis and scoring techniques. Scoring technique using the Fekrat scoring method, by classifying the environmental disclosures into four categories with a total of disclosure items aspects of the environment are eighteen items (environmental control equipment and facilities; past and current operating costs of environmental control equipment and facilities; estimated future expenditures for control equipment and facilities environment; future operating costs of environmental control equipment and facilities; funding for environmental control equipment or facilities; current litigation; potential litigation; comprise air emission information; water disposal information; solid waste disposal information; control, installation, facility, or process explained; facility compliance status; regulations and requirements; environmental policy or company concern for the environment; conservation of natural resources; rewards for environmental protection; recycling; and departments or offices for pollution control). The valuation model of Fekrat et al. [25] to assess aspects of environmental accounting disclosures. Four categories were disclosed i.e. regarding accounting and financial factors, environmental litigation, prevention of environmental pollution, and other aspects [26].

This environmental disclosure calculates that each item of environmental disclosure in the annual report is given a value of three if the item is discussed in monetary or quantitative terms, a value of two if the item is disclosed with company-specific information in non-quantitative

terms, a value of one of the item is only publicly disclosed, and zero value if the item is not in the disclosure [27].

3 Results

3.1 Accounting and financial factors

The first aspect in the Fekrat model is assessing the disclosure of accounting environment companies related aspects of finance and accounting related to the prevention, reduction, and cessation of environmental impact. Disclosure information items consist of past and present expenses for: a = environmental control equipment, and facilities, b = past and current operating costs of environmental control equipment and facilities, c = estimated future expenditures for control equipment and facilities environment, and d = future operating costs of environmental control equipment and facilities, and funding for environmental control equipment or facilities. The following are the results of environmental accounting disclosure scores related to accounting and financial factors in Table 2.

The average disclosure of accounting and financial accounting aspect is zero, which means that the majority of companies in Indonesia are not yet revealing aspects of this. The average zero does not mean that the whole company has not been doing the disclosure, but there are a few companies that have made disclosures and most have not made disclosures. The results showed that the items that have not been widely disclosed are items "a" through "d".

Table 2 Average factor disclosures for accounting and financial factors

Disclosure item	Sector							
	1	2	3	4	5	6	7	8
a	0	0	0	0	0	0	0	0
b	0	0	0	0	0	0	0	0
c	0	0	0	0	0	0	0	0
d	0	0	0	0	0	0	0	0
e	2	1	2	2	1	1	1	0
Total average	0	0	0	0	0	0	0	0

Source: author's calculating

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This shows that many companies have not specifically calculated and revealed the costs and costs that must be incurred for the operation and provision of environmental control facilities. These companies may have incurred expenses related to the environmental consequences of their operating activities but these expenses are not classified in the operating accounts and environmental control facilities. In PSAK itself there is no obligation to classify the account. However, in the ongoing report, all expenses and costs related to the environment must be specified in their designation.

In addition to not disclosing the costs and expenses that have been incurred, many companies also have not been able to budget or estimate the costs and expenses of environmental control. This is indicated by the average of items "c" and "d" which show zero values. This can indicate that the average company has not been able to identify the environmental impact of the company's operations so that it has not been able to determine the operating costs and burdens and facilities needed for environmental control.

Table 3 Expenditures on PT Timah Tbk's costs related to environmental aspects

No.	Program	Amount (IDR)
<i>Waste, Emission and Remediation Management Costs</i>		
1	Waste management	1,750,000,000
2	Emission and air quality monitoring	1,668,725,000
3	Permit protection and environmental management	244 043 .000
4	Life environment development	143,325,000
5	Equipment expenditure, maintenance and operation	1,187,200,000
<i>Cost of Environmental Prevention and Management</i>		
1	Environmental education and training	628,980,000
2	Reporting and documentation of the RKL-RPL and B3 waste program	32,736,000
3	Environmental document consultant	5,571,710,000
4	External environmental audit	412,038,000
Total		11,638,757,000

Source: author's calculating

The item that has been widely disclosed in item "e", i.e. referring funding for environmental control activities and facilities. However, the companies disclose those items in general disclosure and specific disclosure but qualitatively (with an average of one and two). These companies only state that they have carried out activities environmental-related activities in their sustainability report. This form of funding is generally presented in CSR policy statements or in the reports of the board of commissioners and directors.

Companies that have revealed points "a" and "b" generally present in a variety of ways because there are no standards on which to disclose them. For example, PT Indocement Tungal Prakarsa Tbk only reveals that in 2021 the company issued a charge amounting to IDR 21 billion to carry out activities related to the environment. Different PT Timah Tbk (TINS), which is the consumer goods industry, detailing environment expenditure and funds (see Table 2).

Disclosure made by PT Timah presented points "a" and "b" in the aspects of accounting and finance, i.e the costs,

operating costs, and environmental control facilities. Table 3 highlights expenditures from these companies for environmental aspects that are more inclined to cost of environmental prevention and management reaching IDR 6,645,464,000 (57.1%) rather than waste, emission, and remediation management costs of IDR 4,993,293,000 (42.9%). As for the expenditure of PT Timah Tbk based on the highest element is to hire a consultant in making environmental documents up to IDR 5,571,710,000.

3.2 Environmental litigation

The next aspect is the aspect of environmental litigation that assesses whether the company has revealed legal issues that occur now or that might occur in the future. The items assessed are: a = current litigation and b = potential litigation (which may occur in the future). The following are the results of environmental accounting disclosure scores on accounting and financial factors from Table 4.

Table 4 Mean disclosure score of environmental litigation factors

Disclosure item	Sector							
	1	2	3	4	5	6	7	8
a	0	0	0	0	0	0	0	0
b	0	0	0	0	0	0	0	0
Total average	0	0	0	0	0	0	0	0

Source: author's calculating

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The average disclosure of environmental factors on the company's litigation in Indonesia also still shows the number zero. This means that the company has not revealed any legal issues related to the environment that are currently running or that might occur due to environmental problems resulting from the company's activities.

The disclosure also needs to be done if the company is not in the litigation process. This is important for decision-makers because it is related to company contingent obligations (PSAK 31). The company must be able to identify and disclose contingent liabilities resulting from the litigation process related to the environment.

In addition to revealing aspects of ongoing litigation, the company must also be able to conduct analysis and identification of the possibility of potential litigation related to environmental issues. This can be the basis for companies to reduce risks that result in environmental-related litigation. The results showed that none of the companies in the study sample revealed quantitatively the existence of litigation processes and the potential for litigation. Companies that make disclosures only do disclosures in general with a score of one and specific but non-quantitative disclosures with a score of two.

One example of a company that discloses environmental litigation is PT Austindo Nusantara Jawa, Tbk from the agriculture industry. In the sustainability report, the company stated that in 2021, the company did not experience sanctions imposed fines for violations against laws or other environmental regulations.

3.3 Reduction of environmental pollution

Another assessment factor is disclosure related to reducing the environmental pollution. The items is: a =

consist of air emission information, b = water disposal information, c = solid waste disposal information, d = control, installation, facility, or process explained, and e = facility compliance status. *Table 5* highlights the environmental disclosure score related to the reduction of environmental pollution in which the average of all industries expresses the reduction of environmental pollution in general.

The results showed that quite some companies revealed a reduction in environmental pollution even with an average score of one. The average value of the disclosure indicates that the company is still expressing environmental pollution reduction factors in general disclosure. Examples are TINS revealed the company's efforts to reduce environmental pollution by controlling emissions through the lawyer turns to use Cloro Fluoro Carbon (CFC) into hydrocarbons environment friendly gradually along with the business of waste management.

Several companies have specifically expressed pollution reduction, but qualitatively. PT Sampoerna Agro Tbk (SGRO issuer code), in the agriculture industry, explains how activities in waste control are based on the 3R (reduce, reuse, and recycle) policy. Also, the company manages greenhouse gas (GHG) emissions in areas with high carbon reserves using three methods according to the needs, namely RSPO (Ton CO₂e / Ton FFB), ISPO (kg CO₂e / kg FFB), and ISCC (kg CO₂e / Dry Ton FFB). PT Bakrieland Development Tbk (ELTY issuer code), the property industry, real estate, and building construction, shows how to use energy and water consumption each year. Also, the company explained how waste management activities with the 3R policy and working with parties who manage waste into useful products.

Table 5 Average disclosure scores for environmental pollution reduction factors

Disclosure item	Sector							
	1	2	3	4	5	6	7	8
a	1	1	1	0	0	1	1	0
b	1	1	1	1	1	1	1	0
c	1	1	1	1	1	1	1	1
d	1	1	1	1	0	1	1	0
e	1	1	1	1	0	1	0	0
Total average	1	1	1	1	0	1	1	0

Source: author's calculating

However, the most not disclosed points are items "a" and "e", which are related to air emission information and facility compliance status. In item, most of the companies that did not disclose were various industries, the property industry, real estate, and building construction, as well as the trade, service, and investment industries. This is possible because most of these industries do not have a direct impact on air emissions.

Item "e" relates to compliance with the company's operational technical activities related to the use of pollution reduction facilities. In this case, many companies do not disclose whether pollution reduction procedures and

facilities have been carried out according to procedures. Item "e" associated with item "d", which is an explanation of the control, installations, facilities, or processes. In the property, real estate, and building construction industry and the trade, service, and investment industries, on average there is no disclosure of item "d" and item "e". when the company does not have and does not disclose any explanation referring pollution control, installation, facilities or processes, it also tends not to disclose the item's compliance status of the facility.

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3.4 Other aspects

The last aspect of the Fekrat model is another aspect that does not belong to the previous aspect group. The items consist of discussion of: a = regulations and requirements, b = environmental policy or company concern for the environment, c = conservation of natural resources, d = rewards for environmental protection, e = recycling, and f = departments or offices for pollution control. Table 6 also summarizes environmental accounting disclosure scores related to other aspects.

The average disclosure of other aspects shows an average of one. That means companies in Indonesia have revealed items in other aspects even if only in general. Item related to the existence of regulations and requirements from companies related to the environment has not been widely disclosed by companies in the agricultural industry which shows an average of zero. This shows that most agricultural industries do not have or do not disclose the existence of regulations or requirements referring the environment.

Table 6 Average disclosure scores of other aspects

Disclosure item	Sector							
	1	2	3	4	5	6	7	8
a	1	0	1	1	1	1	1	1
b	2	1	2	2	2	1	1	1
c	1	1	2	1	1	1	1	1
d	1	2	1	1	1	1	0	0
e	1	1	0	1	1	1	0	0
f	0	0	0	0	0	0	0	0
Total average	1	1	1	1	1	1	0	0

Source: author's calculating

Item "d" regarding awards for environmental protection has not been disclosed by the company in the infrastructure, utilities, and transportation industries as well as the trade, service, and investment industries. This is related to the relatively low average disclosure of environmental aspects in these industries so that these companies do not yet have an award scheme related to environmental control. At item "e" relevant recycling companies with no disclosure is in the mining industry, agriculture, infrastructure, utilities, and transportation and industrial trade, services, and investment. That is because in these industries it is not possible to recycle the resources that have been utilized.

Disclosure items that have a very low disclosure rate are items related to the existence of a special department for environmental control. The average company in all industries does not have or have not revealed the item. An example of item "a" is at PT United Tractors Tbk (the issuer code UNTR), the industry trade, services, and investment, explaining that the company is committed to carrying out operations without violating laws and regulations in the environmental field. The company conducts regular environmental audits of operational installations and evaluates performance specifically those that have a potential impact on the environment. Referring natural resource conservation, the company conducts Integrated Green Village (KHT) and Green Open Space (RTH) program activities.

4 Discussion

The results showed that basically, companies in Indonesia have revealed environmental accounting even with fairly low average disclosures. This low assessment is because of the total of the average disclosures in all aspects

of the disclosure there is an industry that shows zero value. In this case, zero means that there are quite a lot of companies in the industry that do not make disclosures, namely in the property, infrastructure, and trade industries. We presented the average total disclosures in Table 7.

The results also showed that a total average company has made disclosures related to aspects of pollution reduction and other aspects (related to environmental policies, awards, environmental agencies, conservation, and recycling). The average total disclosure indicates the value of one which means companies tend to only make disclosures in a general form.

This study uses a valuation model used by Fekrat to assess the disclosure of accounting for corporate environments. In his research, Fekrat did not justify the high or low disclosure made by the company. However, the scoring method has scoring numbers 0, 1, 2, and 3 that can be assumed that the company which has an average of zero disclosures (no disclosures) has a lower value than the value of one (disclosures in general). So it can be concluded that a company or industry with a disclosure average value of one has a disclosure value higher than zero. The results of this study indicate an average disclosure of one, so it can be justified that companies in Indonesia still have quite a low level of disclosure when compared with other countries that have an average value of more than one (two or three).

For example, the practice of applying environmental accounting standards in different countries has significant differences. Especially in developing countries such as Indonesia, Jordan, and other developing countries that are members of social and environmental accounting (SEA), where Saleh & Answerreh [28], Homan [29], and Qian [30] emphasize that the level of awareness of companies in environmental management is not optimal. In its

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application, of course, it is necessary to examine disclosures in environmental accounting with investment considerations and decisions. At the company management level, it has indeed pursued the level of environmental awareness. However, the biggest fear is the lack of qualified accountants who are suitable to apply

environmental accounting and understand the benefits or role of disclosure of environmental accounting. There needs to be a transformation of regulations that highlight the environment. Indirectly, the effect presents actual evidence in enforcing applicable laws.

Table 7 Average total environmental accounting disclosure scores

Sector	FA	L	PR	O	AT	HS	LS
Consumer Goods Industry	0	0	1	1	1	29	1
Agriculture	0	0	1	1	1	29	2
Mining	0	0	1	1	1	31	3
Various Industries	0	0	1	1	1	22	2
Property, Real Estate, and Building Construction	0	0	0	1	0	31	1
Basic Industry and Chemistry	0	0	1	1	1	35	1
Infrastructure, Utilities, and Transportation	0	0	1	0	0	21	1
Trade, Services, and Investment	0	0	0	0	0	23	1
Total	0	0	1	1	1	0	0

Source: author's calculating

For developed countries such as China and the Netherlands, national accounts have included environmental information through the environmental, economic accounting system (SEEA). Pirmana et al. [31] monitors SEEA as a high standard at the international level for monitoring and providing valuable support in environmental and economic sustainability. Issues in developed countries also become a practical focus in providing human resources, availability of funds, and data quality. The key to success in implementing SEEA is capacity building, with a consistent framework of variations in the system. Economic acceleration that is always inclusive needs to pay attention to the potential for the environment as an increasingly serious impact. Carbon emissions and smoke become pollution that contributes to pollution and sustainable development. The primary emphasis is the intensity of disclosure of information that understands environmental accounting gradually through economical means and environmental activities [32,33]. The way to improve the quality of the right environment is the position of the company to disseminate accurate accounting information.

Environmental accounting disclosure is part of CSR which is a direct form of corporate responsibility towards the environment and society. The theory or concept of legitimacy which is the concept that underlies the existence of CSR is also the basis for disclosure of environmental accounting for companies. Legitimacy is considered as a strategic factor in the development and survival of the company by operating following the rules and norms that exist in the community [34]. This is because companies are limited by social contracts with the community so that disclosure related to the environment and society is considered to be able to increase the positive value, acceptance (legitimacy) of the community, and the survival of the company.

The strengthening of this phenomenon is very relevant to previous studies that reviewed people's concerns about

drastic changes in the environment because of the expansion of the activities of companies that had only concentrated on the economy so far. In Africa, America, and Asia, markets for some export commodities such as oil palm plantations threaten environmental damage. High global market orientation, of course, has consequences for climate change [35]. For example, Lupu et al. [36] evaluates land that is increasingly degraded because of the development of the tourism industry, so that it is more considered a magnetic field to attract people from urban areas to villages. As a result, land space is increasingly limited and the level of urbanization is also increasing. Massive invasion will actually reduce the spatial function because the social, economic and cultural order is also high. Interestingly, the investigations of Manea et al. [37] conclude that some European countries that have applied environmental accounting principles have difficulty identifying the number of local economic losses caused by the activities of companies engaged in tourism. Especially for modern artificial tourism, many use land (endogenous) and which should still preserved because of natural heritage but has now shifted to highlight the tourist center. The sustainability monitoring process continues to be pursued through the application of an environmental tax for every tourist who travels.

Environmental accounting disclosure is also related to stakeholder theory which states that the existence and sustainability of a company are influenced by the support of stakeholders. Therefore, companies must continue to strive to get this support, one of which is by always providing environmental disclosures. Environmental disclosure will increase the trust and positive value of stakeholders to the company [38].

This is in line with the opinion of Singer [8] that the main factor driving environmental disclosure is the pressure from stakeholders and regulations. Both concepts are proven from several studies that show that there is a significant influence of environmental accounting

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disclosure on stock performance [39]. Besides, a study related to green accounting shows that there is a positive influence between environmental disclosure and environmental performance with the company's ability to generate profitability of the company [40].

Thus, the company must make maximum environmental disclosure related to the environmental impact of its operating activities. The results showed that environmental accounting disclosures at companies in Indonesia were limited to general disclosures indicating weak pressure from stakeholders and regulations. Under PP number 47 of 2020 regarding "business organizations" should have serious consequences on the recognition, measurement, recording, presentation, and disclosure of accounting related to environmental aspects [41,42]. However, especially for companies that directly manage natural resources, recognition related to environmental accounting on average is still on public disclosure with point one. This is related to the absence of standard rules or standards regarding proper disclosures related to environmental accounting.

Disclosure of accounting and financial aspects, pollution prevention, and other aspects in this study show an average of zero which indicates there has not been much quantitative disclosure. The company has not been able to specifically identify the environmental impact of its operational activities so it has not been able to determine the prevention activities and facilities. Therefore, costs and estimates related to activities and preventive facilities cannot be properly disclosed [43]. This happens to companies that do not directly utilize natural resources, such as in the trading or service industries.

Disclosure of litigation is also a factor that many companies do not disclose. This is related to the company's fear of the influence of bad publicity from the litigation process. The company does not want to be judged problematic because it is in the process of litigation or is considered to have problems with disclosing potential litigation. This is contrary to a study that shows that stakeholders will have a positive value from stakeholders by making appropriate disclosures. The company will be considered to have good transparency if the company discloses all risks of its operational activities.

5 Conclusion and contributions

The study aims to understand and explore standards in environmental accounting in companies listed on IDX in 2021. From these findings, we report that there are bright prospects for companies in Indonesia to have disclosed environmental accounting. However, a systematic dynamic occurred considering that the number of companies that made disclosures was only about 45% of the total issuers. The momentum of average disclosures that is carried out is general disclosures with an average of one point. This shows that companies in Indonesia have not been able to

identify activities and environmental impacts, facilities for handling and preventing these impacts.

With Fekrat's assessment, the average total score of one indicates a low score compared to points two and three when the company discloses certain environmental calculations and is based on monetary or quantitative units. There is no research to show whether quantitative disclosure is considered better than qualitative. In addition, the various ways in which disclosures occur are caused by the absence of standard standards governing disclosures.

We also concentrated on the theoretical steps of academic ideas. Most of the disclosures are for pollution prevention, environmental regulation, environmental policy or company concern for the environment, conservation of natural resources, awards for environmental protection, recycling, and departments or offices for pollution control. Average disclosures related to accounting, financial, and litigation aspects show zero, so the average company has not disclosed these aspects.

The escalation of the environmental accounting concept, which is minimally supported by the government, is certainly our concern. Concrete steps can be seen by transforming elements that involve all components of various economic sectors. Here, exploration provides extensive knowledge involving 44.9% sample of all companies officially listed on IDX. It should investigate comprehensively macro evaluations to focus on a country or region, so this is a significant novelty.

The theoretical steps are concentrated on academic ideas. Most of the disclosures are for pollution prevention, environmental regulation, environmental policy or company concern for the environment, conservation of natural resources, awards for environmental protection, recycling, and departments or offices for pollution control. Average disclosures related to accounting, financial, and litigation aspects show zero, which means that the average company has not disclosed these aspects.

The policy in practice involves the government's attention. Regulations regarding environmental accounting disclosure obligations already exist, but the rules for sustainability reporting related to OJK remain voluntary. Therefore, considering the importance of disclosure of the accounting environment and based on the results of research that still shows low disclosure by companies in Indonesia, policymakers in Indonesia must assess the effectiveness of regulations related to environmental protection efforts. In addition, the results of this study indicate the need for the legitimacy of the IDX as the administrator of the capital market to regulate company obligations related to environmental accounting disclosures in its sustainability reports.

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POSSIBILITIES OF THERAPY IN THE DENTAL AREA WITHOUT THE USE OF DENTAL PROSTHESES - REVIEW

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Keywords: regenerative medicine, stem cells, regenerative therapy, dental stem cells, maxillofacial defects.**Abstract:** Dental regeneration therapy opens up access to the provision of biocompatible and living functional tissues, in contrast to current therapies based on prostheses and implants. The identification of dental stem cells has supported research and the effectiveness of therapies for dental defects. For maxillofacial defects after various tooth extractions, traumas and other conditions caused by periodontal diseases, categorized grafts, such as autografts, allografts and xenografts, are also used to regenerate lost bone.**1 Introduction**

Regenerative medicine (RM) is considered a rapidly evolving field of research that encompasses industries such as stem cells, tissue engineering and cell transplantation. Its goal is to replace damaged or lost tissue by acquiring stem cells and to provide new disease therapies. The main component of RM is tissue engineering, which uses knowledge from cell transplantation, materials science and biomedical engineering to develop biological replacements. The role of these replacements is to restore and maintain the normal function of damaged tissues and organs. RM uses several approaches to regenerate disrupted tissues, using injectable cell therapy to regenerate and restore tissue structure, or the use of biocompatible materials to create tissues and organs [1].

In the case of survival of serious trauma or illness, transplantation is in most cases considered necessary to save the patient. As efforts are currently being made to supplement classical transplantation with tissue and organ regeneration, research and application of RM is gaining prominence [2]. The regeneration process is based on the potentials of human stem cells, which arise from different cell types and are capable of self-renewal under appropriate conditions. Regeneration involves the replacement and reconstruction of missing or lost tissue with new ones that will take care of and ensure its proper biological functioning [3].

2 Stem cells

Stem cell (SC) research makes it possible to understand and explain the complex processes that take place in cells, as well as the development of organs themselves. Stem cells are undifferentiated cells that are capable of self-renewal and can become different types of specialized cells. Due to their properties, they are used in tissue regeneration, drug development, toxicology prediction and cell therapy. These cells are present in human body from the embryonic to the adult stage of life [4].

They represent the basis of organs, tissues, the whole organism and among their role during life is to repair and regenerate damaged tissues after an injury or illness. If SCs are genetically modified, they can provide faster spread and anchorage in the body [5].

The properties and behavior of these cells are largely influenced by the environment in which they are located, the so-called "niche". It is a specific microenvironment that is affected by a combination of growth factors, extracellular matrix and mechanical and chemical stress. It helps maintain and regulate the balance between cell differentiation and self-renewal. The process of differentiation is the gradual specialization of cells based on their structure, function and interrelationships during the development of the organism. Great efforts are currently being made to determine how the individual components of this environment affect stem cell function.

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Innervation can play a major role in the functioning of these cells by affecting the regulatory sequence of events. Studies have shown that parasympathetic nerves regulate progenitor cells and are essential for salivary gland regeneration and development. The SC study itself will determine the direction in which regenerative medicine will go.

In dentistry, one of the most important experiments is the cultivation of a functional tooth with the help of stem cells. This process would be considered useful especially in congenital conditions where the tooth is completely missing or has not developed in part.

It will be able to be used as a regenerative therapy especially when technology and research progress to a state where the tooth can grow within months, not years. Stem cells should be useful and useful in the regeneration of dental pulp, periodontal ligament and bone [6].

Some research has shown that soft tissue regeneration and craniofacial muscle reconstruction are more likely to succeed in the future than tooth regeneration. These cells can be classified into several main groups, according to their differentiation potential and origin (Table 1).

Table 1 Classification of stem cells according to potential and the possibility of their differentiation

Cell type	Differentiation
Totipotent	Create any other cell type (zygote during fertilization)
Pluripotent	Source of almost every cell type (derived from three germ layers)
Multipotent	They form several cells in the body
Oligopotent	Produce more cell types (lymphoid and myeloid)
Unipotent	Create one cell type (muscle cells)

2.1 Dental stem cells

Dental stem cells (DSCs) are most similar to mesenchymal stem cells (MSCs) in their nature, i.e. in their ability to self-renew and differentiate. Dental pulp stem cells (DPSCs) were first isolated, and other cells were later discovered. These include human exfoliated tooth stem cells (SHED), periodontal ligament stem cells (PDLSC), apical papilla stem cells (SCAP) and dental follicle stem cells (DFSC) (Figure 1) [7].

DSCs can be isolated from the dental pulp of deciduous and permanent teeth, as well as from the periodontal ligament and apical region of the teeth, as well as various other structures of the healthy tooth.

According to their origin, they originate from mesenchymal cells and neural crest cells. The odontoblasts that line the inner surface of the tooth come from MSCs and are involved in the production of dentin. Enamel-forming ameloblasts are responsible for the formation of dental tissue. These ameloblasts belong to the epithelial cells of the dental lamina [8].

The SHED and DPSC studies found a significantly higher rate of proliferation and gene expression in SHED.

Given the demonstrated capabilities, this demonstrates that SHED could be an option for therapeutic applications [9].

An important factor is also the study of innervation in tooth regeneration and the impact on tooth stem cell populations. Another aspect of regeneration is to ensure the vascularization of the teeth, because the dental pulp is richly vascularized and the teeth are dependent on the supply of nutrients and oxygen to the blood. While blood vessels are found in the pulp of the tooth germs in the early stages of development, innervation is formed only in the late stages of odontogenesis. Innervation and vascularization are the basis of the physiology and pathology of the teeth, identification of the mechanisms that regulate these processes will be essential in the partial regeneration of the marrow, or complete regeneration of the teeth.

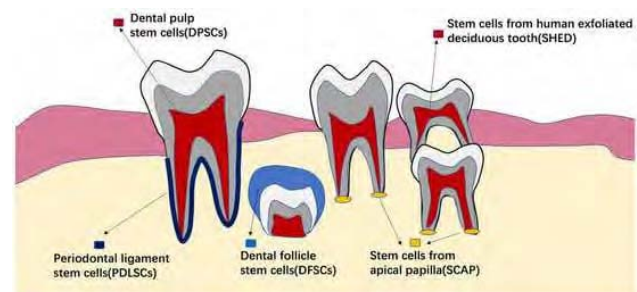


Figure 1 Schematic picture of dental stem cells [10]

3 Possibilities of therapy without the use of dental surgeries

Current dental prosthesis procedures are based on implants and dental replacements, which, however, fail to form a natural root structure, which in turn leads to the loss of supporting bone due to inflammation of the gums and bones around the dental implants. New strategies and therapies for dental regeneration *in vitro* using stem cells, biomaterials, 3D culture conditions that can faithfully mimic the environment in which dental stem cells are located are being explored [11].

3.1 Tooth regeneration

Regeneration of the whole tooth, which is based on similarities to the natural development of teeth, is a complex set of complex processes. It requires precise interactions and repeated molecular signaling between dental epithelium and dental mesenchyme (DM). This mesenchyme originates from neural crest cells, condensing around the dental epithelium to form a dental structure called the dental bud (root) [12].

These dental buds are highly specialized dental organs that consist of dental epithelial and mesenchymal cells that develop into adult teeth. Several approaches to tooth regeneration have been studied and tested, focusing on individual tooth components such as dentin, dental pulp, enamel, cement and periodontium. The combination of

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these studies shows promising results, but does not guarantee successful regeneration of a viable tooth [11].

Research has shown that early stages of embryonic dental epithelium can induce tooth development. In the later stage of tooth development, odontogenic capacity is transferred to the dental mesenchyme, and this induces tooth formation when combined with the epithelium. The ability to regenerate healthy teeth in humans is partially lost because dental epithelium is no longer present in these teeth. Therefore, two approaches have been proposed to restore human teeth. One of these approaches is the formation of tooth root *in vitro* (Fig. 4) and its subsequent transplantation to the defect site. The second possibility is the formation of a bioengineered tooth root at the defect site *in vivo* [12].

A mouse model of embryonic dental buds was studied for tooth regeneration. The layers of mouse dental epithelial (DE) and dental mesenchymal (DM) cells were joined to form an artificial tooth bud. Tooth formation was observed in combination with DE with DM cells, embryonic stem cells from the spinal cord and bone marrow.

In 2007, Nakao et al. has seen the successful regeneration of fully functional teeth using artificial embryonic cell embryos. In the following years, this method proved to be functional and is an advanced model for the functional regeneration of teeth from embryonic tissue. Single-cell DE and DM were harvested from embryonic embryos of mouse teeth and cultured *in vitro* to develop into an early stage. They were then transplanted into the mouse jaw. This study reports full-size tooth formation [12,13].

The scheme below (Figure 2) shows cells taken from embryonic enamel and pulp, the tissues are subsequently recombined in a collagen gel drop and cultured *in vitro*. The restored dental embryo is then transplanted and grown in the jaw bone of the host adult animal.

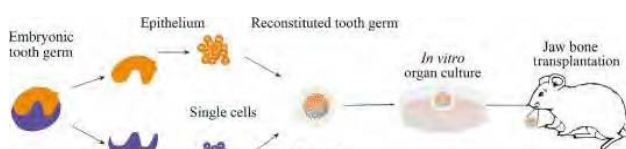


Figure 2 Method of whole tooth regeneration by *in vitro* culture and subsequent transplantation to the defect site [12]

Another possible therapy for tooth regeneration is the possibility of using induced pluripotent stem cells (iPSC). The researchers differentiated mouse iPSCs into cells similar to the neural crest and odontogenic mesenchymal cells. They further reprogrammed the patient's somatic cells to a mesenchymal line derived from the ectodermal epithelium and neural crest. This recombination of the cells and the subsequent transplantation allowed the formation of a dental embryo and a functional adult tooth. Many factors need to be taken into account in the complete regeneration of a functional tooth, but the use of iPSC seems to be a promising path. These cells have optimal

proliferation and higher potential in autologous transplantation than other cell types. When combined with biomaterials and different types of scaffolds, iPSCs can induce and promote tooth development [11].

3.1.1 Usage of scaffolds and biomaterials

Dental tissue engineering relies on a combination of elements such as cell, scaffold and regulatory mechanisms (biological environment) for functional and proper tissue regeneration. Scaffolds and biomaterials are an important part because they support this regeneration. In order to function properly, they must meet certain requirements for the *in vitro* physiological environment necessary for cell growth, expansion, and differentiation. Scaffolds must meet biocompatibility conditions, have porous structures that allow cells to penetrate and must also ensure nutrient diffusion. They should be able to prevent chronic inflammatory diseases and provide regulatory signals to promote healing. Biocompatible and biodegradable materials have been developed and optimized over the years to support the regeneration process. Natural biomaterials, also referred to as nature polymers (collagen, keratin, chitosan, platelet-rich plasma, etc.), have low toxicity, are environmentally friendly and are a cheaper choice than synthetic biomaterials. Their advantage is that they promote cell adhesion, proper cell signaling and degradation without immune rejection. Synthetic materials such as polylactic acid, polyglycolic and polycaprolactone are more elastic and flexible than natural ones, but they are not as favorable for remodeling as natural ones. Composite materials are most preferred. Thanks to the development of these biomaterials for scaffolds, tissue engineering in dentistry has made significant progress [11].

3.2 Gene therapy

Gene therapy currently appears to be a promising therapy option in regenerative medicine and tissue engineering. It presents options such as circumventing the limitation of the short half-life of growth factors *in vivo* and controlling protein distribution. It uses genetically modified cells to deliver specific doses of bioactive protein over a long period of time. Gene therapy involves several steps, such as consideration of tissue growth factor, route of administration, target cells, and local effect. Non-viral plasmids, small circular DNA structures, can replicate in the cell independently of chromosomes. They are considered safer for the host than viral vectors because they are not incorporated into chromosomes. They provide a protein expression transition and improve bone formation with various carriers [14].

In a study by Dunn et al. recombinant adenoviral vectors encoding the bone morphogenetic protein BMP 7 were delivered to the bone defects using a collagen matrix. The results revealed that this combination led to improved alveolar bone defect filling and new bone contact with the implant, and thus *in vivo* BMP-7 gene therapy offers potential for alveolar bone engineering applications [15].

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Combination gene therapy using AdBMP7 alone or with an adenovirus with a mineralization protein gene (LMP3) promoted bone formation *in vivo* through progenitor cells. Adeno-associated virus (AAV) has a single-stranded DNA genome and requires the host DNA polymerase to form a complete strand. In periodontitis, AAV inhibited disease progression and prevented alveolar bone loss. Gene therapy is performed by various techniques. MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of a target gene. They affect osteogenesis, or the regulation of genes involved in direct bone formation. MiRNA research in bone development is based on *in vitro* cell culture experiments, but there are *in vivo* studies that demonstrate promising results in bone repair [14].

3.3 Dental autotransplantation

Dental autotransplantation is a surgical procedure consisting in the replacement of missing teeth, especially in minors and young adults, where implants or fixed prosthetics are not a suitable solution for various reasons. This treatment can be defined as the transplantation of a tooth from its original position to a surgically prepared recipient site in the same patient. An autotransplanted tooth, unlike an implant, allows alveolar growth along with adjacent teeth and periodontal ligament. It has the potential to induce new bone, gingiva and periodontal ligament formation at the recipient site. An important factor influencing the success of this transplant is the presence of the periodontium and its support. There must be sufficient bone and adequate width of keratinized tissue at the recipient site. The problem of achieving periodontal and marrow healing of the transplanted tooth is proving to be a biological limitation. Transplantation should be performed when they have formed from the root of the donor tooth and this statement limits the age range of the transplant. High survival rates are reported in studies mainly in the autotransplantation of premolars, ie the front chairs. Before planning surgery, the patient must meet certain indicators of how to fall into the younger age category and must have the presence of a suitable donor tooth for transplantation. Missing tooth therapy is performed by implantation and prosthetic treatment, but this option is not suitable for young patients who are still developing the body. Here, autotransplantation, which is also referred to as a biological prosthesis, is a suitable therapy [16,17].

Case studies have been published in a review article by Tsukiboshi and colleagues on the long-term outcomes of dental autotransplantation. In a study in a 17-year-old patient, resorption (hard tissue loss) of the mandibular left second molar was observed (Figure 3A). This molar was replaced by autotransplantation of the adjacent left third immature molar, which was considered the best option in this situation. After 12 years of follow-up, successful healing of the periodontal ligament and pulp was noted, and root development was likely to be arrested.

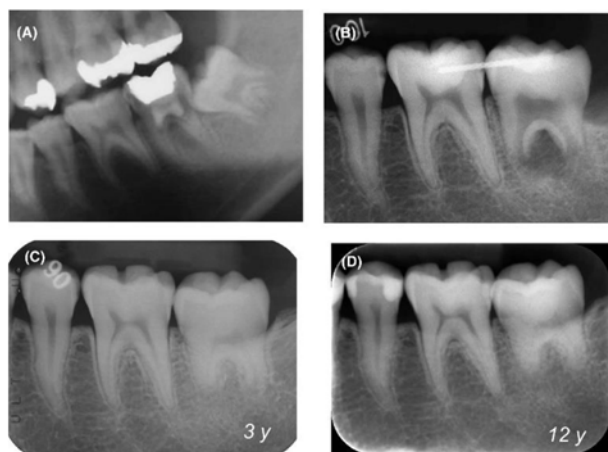


Figure 3 External root resorption (patient 17 years). (A) - view around the site of the procedure; (B) - X-ray after transplantation; (C) - three years later (positive electrical pulp testing, low root development); (D) - twelve years after autotransplantation [55]

In another case of a 39-year-old patient, the left mandibular molar (Figure 4) was replaced by a third mandibular molar due to its unfavorable prognosis. Because the replaced tooth had complete root formation, root canal treatment began two weeks after autotransplantation and was completed after four weeks. The transplanted tooth was followed for 30 years and did not show any associated complications, its normal function was maintained [18].

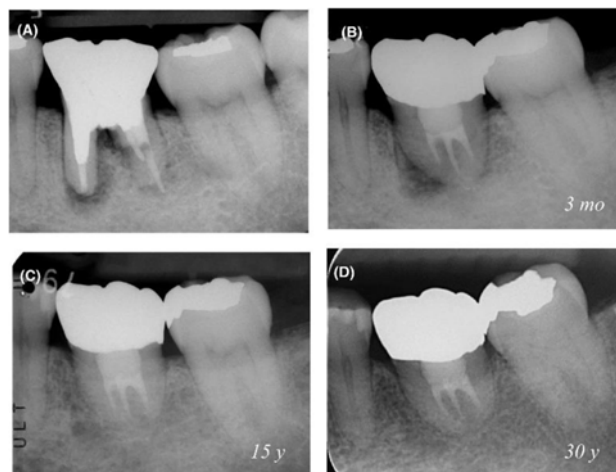


Figure 4 Patient 39 years old. (A) - X-ray before treatment; (B) - 3 months after autotransplantation and crown production; (C) - 15 years after follow-up; (D) - 30 years after surgery [18]

3.4 Gene therapy

The very term regenerative endodontics was adopted in 2007 by the American Endodontist Association based on an understanding of the concept of tissue engineering. Endodontic regeneration applies stem cells, scaffolds, tissue engineering, bioactive growth factors at the canal site to regenerate pulp tissue that has been damaged by trauma, infections, or congenital anomalies. Permanent

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immature teeth with necrotic pulp are usually treated by apexification, where calcium hydroxide is used to induce the formation of an apical hard tissue barrier before filling the root canal. However, this procedure does not have the potential to repair damaged tissue at the canal site and promote thickening and maturation of the root canal walls. That is why a new therapy and treatment option has been introduced under the term revascularization to manage an immature permanent tooth with apical periodontitis [19].

The advantage of regenerative endodontics is the revitalization of the tooth, but also the subsequent development of the roots and increased resistance to fracture. It consists of three steps: disinfection of the root canals, induction of bleeding to form a scaffold, ie the carrier for the cell stem, and coronal sealing of the blood clot with biocompatible material [20].

In experimental studies by Nygaard-Ostby (1961) and Nygaard-Ostby & Hjortdal (1971), they attempted to induce bleeding from periapical tissues into the canal space of the teeth, which was partially filled with root filling. Histological examination of the extracted teeth revealed that connective tissue and cellular cement subsequently formed in the apical canal, which contained the vital marrow. However, no regenerated tissue was formed in the apical canal space in the necrotic marrow teeth. Iwaya et al. consulted the first group to apply revascularization treatment to immature permanent teeth with apical periodontitis. The concept was based on experiments in the revascularization of reimplanted and autotransplanted dog teeth, as well as on the disinfection of root canals. In 2004, Banchs & Trope added the antibiotic minocycline to the antibiotic used by Iwaya, and the treatment became known as triple antibiotic paste. This procedure led to the elimination of clinical symptoms of apical periodontitis, as well as to the promotion of thickening of the canal walls. Subsequently, this regenerative therapy was recommended as a suitable alternative to the traditional apexification for immature permanent teeth with necrotic marrow [19].

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Murray et al. defined regenerative endodontics as "biologically based procedures to replace damaged dental structures, including dentin and root structures, as well as pulpentin complex cells" [21].

3.4.1 Revascularization and regeneration

Revascularization has been mentioned in studies in the healing of pulpal wounds after replantation of immature permanent teeth. As mentioned above, the term was first used by Iwaya and colleagues. Treatments included sodium chloride lavage along with an intracanal antibiotic paste. The result was the elimination of clinical symptoms

and remission of apical periodontitis, thickening of the canal walls and development of roots. Induction of periapical hemorrhage into the canal space is considered a necessary step in regenerative endodontic procedures, and it has been suggested that blood clots in the canal space could serve as a scaffold to promote healing of soft pulp tissue. In a study carried by Lovelace et al., they showed that provoked periapical hemorrhage brought mesenchymal stem cells into the canal space. Blood contains many growth factors that are derived from platelets, so periapical hemorrhage brings the fibrin skeleton, bioactive growth factors, and mesenchymal stem cells to the canal site. Growth factors embedded in the dentin matrix after demineralization of the dentin by rinsing ethylenediaminetetraacetic acid in endodontic procedures are also released [20,22].

Mesenchymal stem cells in the apical papilla of immature permanent teeth with necrotic pulp, which are introduced into the canal site during endodontic procedures, could be able to differentiate into odontoblasts and dentin production. Hertwig's epithelial root canal is able to signal these cells in the dental follicle to differentiate into cementoblasts and thus regulate root development. Histological studies of teeth with necrotic pulp and apical periodontitis after regenerative therapy revealed that the tissues formed were similar to cement, bone, or periodontal tissue. In many animals, it was not a matter of requiring marrow tissue. Wall thickening or root maturation was caused by the deposition of cement-like tissue or bone on the canal walls. In another study, the formation of nerve fibers in newly formed tissue in the duct space of a revascularized tooth was demonstrated [22].

Clinical examinations in a specific study by Nosrat et al. were targeted at two patients aged 9 years (female) and 10 years (male) and showed asymptomatic results after regenerative endodontics. X-rays after four months showed root maturation in both patients. The roots showed a well-developed layer of dentin surrounded by periodontal ligament at the outer edges of the root. The MTA material was recorded in the coronal parts of the tooth and a layer of hard tissue was observed beneath it. No inflammatory cells were found in the canal spaces. In a 10-year-old patient (Figur 5), parts of the roots were surrounded by dentin at the outer edges of the root and the primary dentin layer in the canal spaces was preserved. A much larger layer of dentin was observed in this tooth than in another tooth. The walls of the root canal were covered with a layer of repair cement mixed with osteoid tissue.

The patients developed roots (Figure 6), which is considered a sign of success after regenerative endodontic treatment. However, histological reports indicate that the tissue formed in the root canal is not true marrow tissue. It resembles periodontal tissue and the hard tissues on the walls of the dentin are similar to cement or bone tissue [23].

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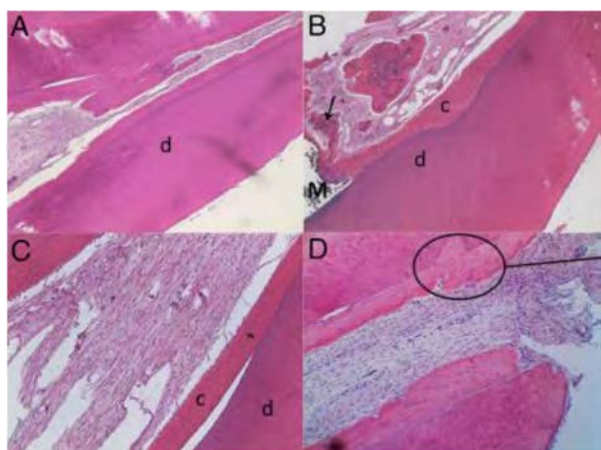


Figure 5 Histological images of a 10-year-old patient. (A) the presence of primary dentin (d) surrounding the canal space. (B) A layer of mineralized tissue is formed under the MTA (M). (C) the presence of repair cement (c) on the walls of the dentin. (D) cement ingrowth on dentin walls [60]

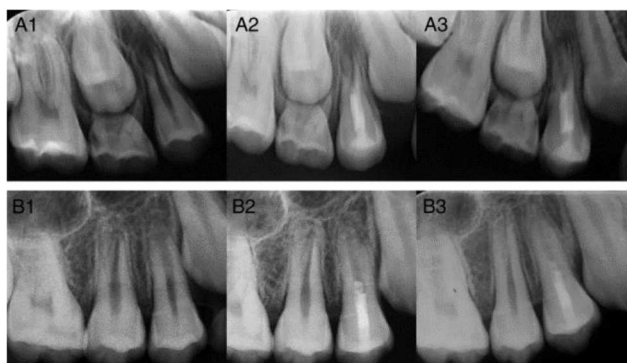


Figure 6 Root development in both patients [23]

Cohort et al. investigated 20 cases of regenerative endodontic treatment with necrotic teeth, where 100% success rate or survival rate of treated teeth in terms of remission and disappearance of apical periodontitis or tooth retention was demonstrated. Coagulation of the canal walls or root development in these necrotic pulp teeth is unpredictable. Thus, it has been shown that this therapy could be a suitable alternative to non-surgical root canal therapy in terms of elimination of clinical symptoms and disappearance of periodontitis. Regenerative endodontic therapy is not suitable for all teeth with necrotic pulp. If the primary goal is to achieve the disappearance of periodontitis, then this therapy can be used, but it is not a suitable alternative for teeth that require a coronary restoration pin [22].

3.5 Regenerative periodontal therapy

Improving the survival of teeth and reducing the progression of periodontitis are provided by reconstructive periodontal interventions, which also provide stability of the result in the long term. Natural teeth provide better long-term survival and marginal bone changes compared to dental implants. Periodontal reconstruction is a complex biological process that involves the formation of lost dental

structures, including alveolar bone, cement, and periodontal ligament on a previously diseased root surface. These reconstructive procedures have advantages over conventional surgical procedures in terms of long-term stability, improved tooth survival, less progression of periodontitis, and less need for long-term interventions. Periodontal regeneration can be achieved by applying barrier membranes, grafts, wound healing modifiers and their combination [24].

3.5.1 Guided tissue regeneration and enamel matrix derivative

Guided tissue regeneration (GTR) mechanically isolates the defect and is based on the application of a separation barrier membrane. This approach based on biological and mechanical concepts has been shown to be successful in both clinical and preclinical studies, but several shortcomings have been reported. These are complications caused by membrane exposure and recession of adjacent teeth, shortcomings in the treatment of several proximal defects and incomplete adaptation of the tooth by the membrane around asymmetric roots. Enamel matrix derivatives (EMD) are the most evaluated in clinical and preclinical models. It is a biologically active compound which, when applied to the exposed root surface, triggers a process of biological processes. This process increases the migration of mesenchymal cells and their attachment to the root surface, as well as their differentiation into cementoblasts, osteoblasts and fibroblasts.

EMDs increase gene expression, which stimulates protein and mineralized tissue synthesis in PDL cells. The process itself later leads to the reconstitution and regeneration of the periodontal apparatus. The use of EMD during therapy improves bone formation [24].

No significant differences were found when comparing EMD to GTR, but GTR is associated with a greater recession and postoperative complications than swelling. EMD is a good treatment alternative for multiple proximal defects without reducing blood valve nutrition and this leads to extensive membrane exposure. While GTR is known to be controversial in bone loss defects, EMD improves outcomes in these defects. EMD also improves oral wound healing by promoting blood vessel and collagen fiber formation in connective tissue, increasing gingival fibroblast proliferation, and influencing inflammatory and healing responses by various cellular mechanisms with a positive effect [24,25].

Since the separate use of EMD as a therapy can be used mainly for narrow defects, combination therapy is also used. It focuses on various alternatives to periodontal reconstruction and includes regenerative principles such as conductivity and inductance, matrix development and cell differentiation, as well as wound space and stability. The combination of biomaterial grafts, biological agents together with EMD reduces postoperative recession.

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Combination therapy includes the application of autogenous connective tissue grafts using EMD.

Histological reports in humans have shown the formation of new cement, bone and adhesion. EMD has a positive effect on inflammatory reactions and enhances the effect on fibroblasts, thereby increasing proliferation. The study by Nemcovsky and Beitlitum includes a series of cases where a modified approach to the treatment of lower periodontal incisors was performed. In all cases, a combination therapy consisting of a single access valve, a conditioned root, an EMD application to the root surfaces, and an autogenous connective tissue graft was used for treatment. The results were satisfactory as there was an increase in the width and keratinization of the gums (Fig. 9), without pulling the bridle and muscles along with the radiographic bone filling of the defects. The results showed that the combination therapy for reconstructive periodontal treatment was able to successfully treat severely affected periodontal lower incisors with gingival recession [24].

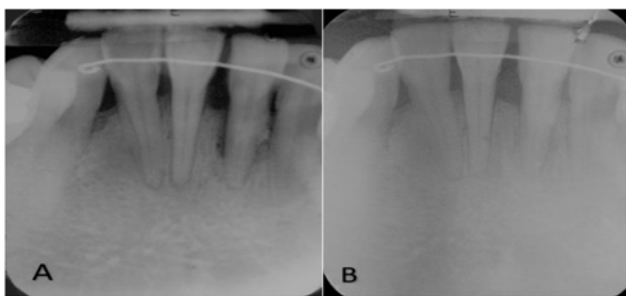


Figure 7 A - before combination therapy (gum recession, bone loss); B - three years after therapy stable results [24]

3.6 Spheroids and organoids

Organoids and spheroids are considered to be new treatments and therapies in dentistry. It is the development and study of stem cells from *in vitro* three-dimensional culture in order to mimic the physiological properties and tissue architecture of embryonic cells. In 2011, Berahim and colleagues grown spheroids from human periodontal ligaments and subsequently transplanted them into collagen- and polyglycolic acid-enriched membranes [26].

They have demonstrated the ability to grow, migrate and proliferate these cells. In 2017, she and the team created a bioengineering tooth (Figure 8) on a dog model that was physiologically similar to a normal human tooth. Through these studies, the authors have shown that the combination of epithelial tissue with mesenchymal tissue, or mesenchymal and epithelial cells provides better tooth formation. By culturing these tissues and cells, they formed a germ organoid, which they transplanted into the dog's jaw, which then developed into a bioengineered tooth with dentin, enamel, and marrow tissue several weeks later [27].

In another study by Jeong et al. developed organoids by culturing DPSCs with matrigel, which were similar to dentin pulp. These characteristics have also revealed the differentiation of odontoblast-like cells, which represents a

promising prospect for the use of these structures in dentistry [27].



Figure 8 Autologous transplantation of a dental germ into the lower jaw of a dog [64]

Other representatives who received a bioengineering tooth were Wang and the team. They combined isolated epithelial and mesenchymal cells to form a dental organoid, and after transplantation into the mouse jaw bone, the organoid later developed into a full-size tooth [28].

Dental organoids and spheroids represent a promising future and an opportunity for advancement in dental procedures. These 3D cultures make available a system for modeling human organogenesis, disease modeling and regenerative medicine. They make complex interactions between cells, the flow of molecules and nutrients available for self-organization to help scientists understand the physiology of the tooth and dental tissues. 3D cultures support the interaction between cells and the macroenvironment, which play an important role in cell migration, proliferation and differentiation [11].

4 Conclusions

As regenerative medicine is a broad area of research that consists of various disciplines and uses knowledge from materials, cell transplantation and biomedical engineering to develop biological replacements, dental therapy research can also be considered a leader in this field. Whereas in dental medicine they focused mainly on the restoration of dental function with the use of implants and prosthetic replacements, nowadays the focus is on the restoration and regeneration of the entire craniofacial area, including the surrounding structures and tissues. The central focus of RM is stem cells, which are able to regenerate and replace damaged organs, soft and hard tissues. Revealing the real cause of defects and diseases in the dental area helps to treat them. The output of this work was to present at the same time therapies based on the principles of RM, whether using SC, scaffolds, biological mediators, or a combination thereof. Although much research and studies are still needed into the potential benefits of regenerative medicine, its benefits and risks, there are now many clinical studies demonstrating the potential of regenerative therapies based on stem cells, biomaterials, scaffolds and growth factors. In the future, RM represents a wide range of uses in treatments and therapies, both in humans and animals.

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PROPOSAL FOR OPTIMIZATION OF BIOMEDICAL FILAMENT PRODUCTION

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Keywords: Laminar box, filament, filament maker, extrusion.

Abstract: Filament production is a demanding process, which we decided to optimize by designing a laminar box in this scientific study. From a number of designs, we decided to choose a purposeful design for the future construction of the laminar box. The production of filaments takes place on a filament maker, which must be placed in a laminar box. The laminar box then provides ideal conditions in the production process, such as the optimum ambient temperature, which according to standards should be in the range of 18 to 19 degrees Celsius. Furthermore, this laminar box is equipped with a thermometer, hygrometer and control unit. All technical specifications are written in this scientific study. The study contains a number of illustrations for a better idea. The laminar box represents a significant contribution to the production and optimization of the conditions for the production of biomedical filaments. His design is unique and follows from the scientific research of the authors of the scientific study.

1 Introduction

The production of filaments can also be characterized as a several-step process (figure 1) from obtaining the material in the form of pellets through drying and extrusion of the filament itself on a filament maker.



Figure 1 Optimized production process

The laminar box can be also defined as a laboratory station designed to work in dust-free, sterile conditions. The use of laminar boxes is broad-spectrum. Laminar boxes are mainly used in applications of optical, laser, semiconductor and electronic technologies. The design of the laminar box is designed to prevent contact with the external environment and thus ensure the protection of researchers as well as the researched material. Most laminar boxes are equipped with a HEPA filter, through which air is sucked in and blown out by a very smooth, laminar flow towards the user outwards. Another important function is to ensure the protection of the product (workspace) against particles. The picture shows a laminar box, which is equipped with two filters, a fan, an air intake

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and an air extraction opening. The construction of this laminar box is simple and practical with transparent walls.

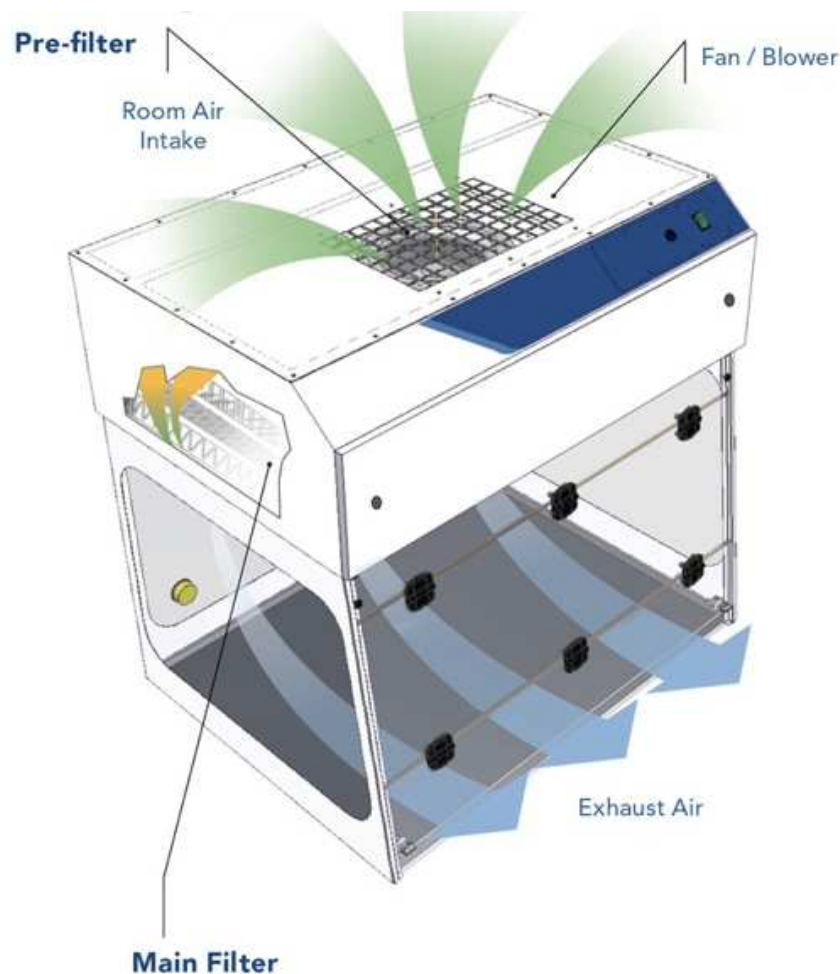


Figure 2 Laminar box [1]

Laminar boxes can be equipped with UV light, which has the task of sterilizing the working environment of the laminar box. The unit with UV light is equipped with a timer and a sensor that prevents exposure to UV radiation when lifting the door [1-6].

1.1 Laminar box design

As part of improving the quality of biomedical filament production, we at the Department of Biomedical Engineering and Measurement proceeded to our own design of a laminar box called Biomedic laminar box. We made the model of the laminar box in the 3D modeling program SketchUp, the interface of which can be seen in the picture. The laminar itself stands out with its simple and practical design (figure 3a and figure 3b). The compact dimensions ensure trouble-free handling when operating the filament maker in the production process. The sterile environment is ensured by insulation. It is made of aluminum profiles and plexiglass with a thorough

connection. Air recovery in the laminar box is ensured by twelve fans, which supply and discharge air.

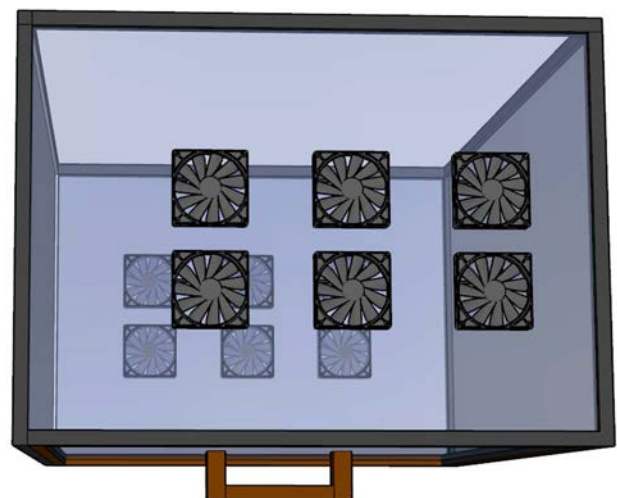


Figure 3a Laminar box design

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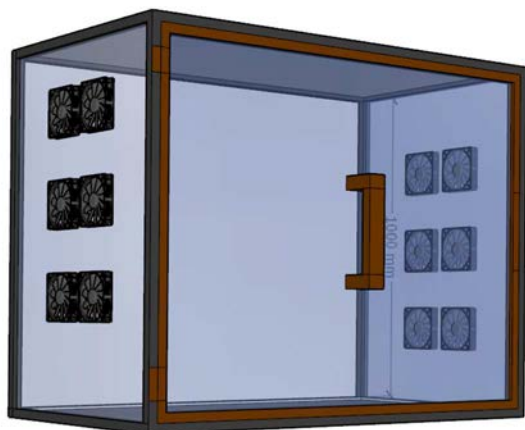


Figure 3b Laminar box design

The interface of the 3D modeling program SketchUp perfectly fulfilled its purpose in the design of this unique laminar box.

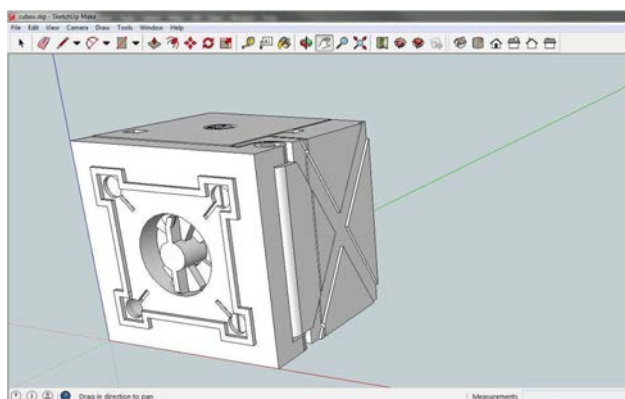


Figure 4 3D modeling program interface

The laminar box is equipped with a thermometer, hygrometer and control unit. Control is provided via the open-source Arduino UNO platform and the Arduino IDE program via a computer. The platform is located in an electrical protection chamber attached to the wall of the laminar box. The side view of the Biomedical laminar box shows (figure 5). This laminar box design serves as a theoretical basis for real construction and optimization of conditions for the production of biomedical filaments.

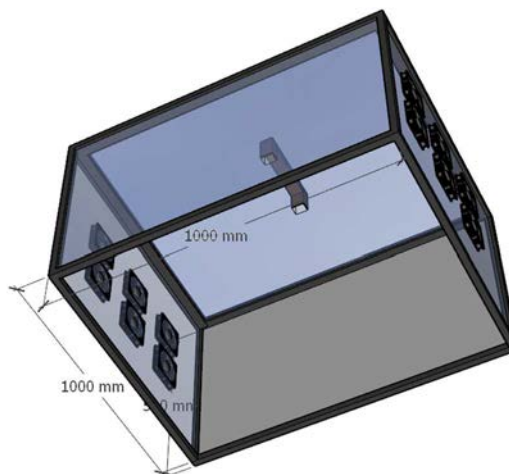


Figure 5 A side view

The table 1 shows more detailed technical specifications and dimensions of the laminar box.

Table 1 Technical specifications

Designation of laminar box	Biomedic laminar box
Width	1000 (mm)
Height	1000 (mm)
Depth	500 (mm)
Air inlet	With 6 power fans
Air outlet	With 6 power fans
Optimum working environment temperature	18-19°C

2 Conclusions

This scientific study has a contribution in the field of material extrusion and provides a theoretical design with real solutions for the construction of a laminar box. At the end of this study, the authors point out the continuation of research. Filament production is gaining more and more prominence. Therefore, the production conditions cannot be neglected. The future of the use of filaments lies in the production of biocompatible implants through additive technology. The study describes technical specifications that indicate success in the production and use of laminar box.

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