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SELF-ASSEMBLED MULTILAYERS OF WATER GLUCOSE MODIFIED-CHITOSAN AND GLUCOSE OXIDASE FOR DETECTION OF GLUCOSE IN MILK SAMPLES

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ABSTRACT

Background: A crucial aspect of electrochemical enzymatic biosensor development is the immobilization of the enzymes, as it directly influences the sensitivity of the bioelectrode. Among the different methods used to incorporate enzymes on the surface of the transducers, layer-by-layer (LbL) self-assembly based on electrostatic interaction with polyelectrolytes of opposite charge stands out due to its simplicity and reproducibility. **Aims**: The aim of the work was to develop an electrochemical glucose biosensor by LbL assembly of a new functionalized chitosan polycation and the enzyme glucose oxidase (GOx). **Methods**: Chitosan was chemically functionalized with glucose by the Maillard reaction. The resulting polycation, named G-Chit, is soluble in the medium compatible with the enzyme. The bioelectrode was obtained by alternating adsorption of G-Chit and GOx onto carbon paste electrodes. By selecting the number of bilayer of G-Chit/GOX, the enzyme concentration, and the pH, the electroanalytical performance of the biosensor was optimized. The electrochemical responses were characterized by cyclic voltammetry and chronoamperometry. **Results:** Under optimized experimental conditions, the biosensor exhibited a sensitivity of (0.81 ± 0.03) µA mM^{-1} in a glucose concentration range of (0.18 to 1.75) mM. **Discussion**: Results indicated that catalytic response increases both with the number of G-Chit/GOx bilayers and the enzyme concentration, obtaining the best responses for 3 bilayers and 2 mg mL^{-1}, respectively, while the optimum working pH value was 7.0. **Conclusions**: The analytical response of the biosensor was tested in milk samples with negligible matrix effects, suggesting a potential application in other dairy products. Results show that G-Chit appears promising for the immobilization of enzymes.

Keywords: *Glucose biosensor, glucose-functionalized chitosan, carbon paste electrode.*

1. INTRODUCTION

Glucose quantification is of significant importance in the chemical and biological industries, in clinical analysis, as well as in food processing and fermentation (Juska and Pemble, 2020; Yusan *et al*., 2018). In the food industry, the glucose content is associated with storage time, fermentation process, and final quality control (Paz Zanini *et al*. 2016). For those reasons, accurate glucose quantification with a quick and simple method has driven the search for new testing devices and procedures. Various methods have been reported for GOx immobilization, such as covalent cross-linking, electrochemical polymerization, sol-gel encapsulating, and layer-by-layer (LBL) self–assembly based on the electrostatic interaction with polyelectrolytes of opposite charge (Bracamonte *et al.* 2014; Ma et al. 2022). This last method has attracted great interest due to its simplicity, wide variety of materials that can be used, and the precise control of the composition and thickness of the layer on the molecular level. Among other polymers, the natural polycation chitosan (Chit), a copolymer composed of randomly distributed units of N-acetyl-D-glucosamine and D-glucosamine linked by β(1→4) bonds, has been employed to immobilize enzymes (Petrucci *et al.* 2021) The biocompatibility, biodegradability, non-toxicity, and bioactivity of Chit, associated with desirable physical and mechanical properties have made this polymer interesting and promising in biosensors development. However, Chit applications are limited because of their poor solubility at neutral or basic pH. Therefore, several studies have been conducted to obtain Chit derivatives soluble in an aqueous medium at physiologic pH (Rocha Neto *et al*., 2022). One of them involves the Maillard reaction (MR) between the amino group of Chit and the carbonyl group of reducing sugar (Arata Badano *et al.,* 2019; Hafsa *et al*., 2021; Yang *et al.,* 2020).

In this work, we used Chit modified with glucose residues (G-Chit) synthesized through MR reaction to immobilize GOx by the LBL self–assembly technique at carbon paste electrodes (CPE). This material has advantages in its low fabrication cost, renewable or disposable electrochemical interface, and low background current (Donmez *et al*. 2017). The electrochemical properties of the bioelectrode have been characterized by chronoamperometry (CA) and cyclic voltammetry (CV). Furthermore, the electroanalytical performance of the biosensor was evaluated for the determination of glucose in standard solutions and milk samples. The presented results confirmed the suitability of this supramolecular assembled bioelectrode for the fast quantification of glucose.

2. MATERIALS AND METHODS

2.1. Materials

Glucose Oxidase (GOx, EC 1.1.3.4 from Aspergillus Niger type II) lyophilized powder containing 17300 units g^{-1} solid. Reagent grade D-(+)-glucose and ferrocene methanol (FcMe) were purchased from Sigma-Aldrich SA (Buenos Aires, Argentina). Reagent-grade buffer phosphate potassium salts KH_2PO_4 and K_2HPO_4 were obtained from Cicarelli. Medium Mw Ch (583 kD) was from Sigma-Aldrich (MO, USA). Analytical grade acetic acid (CH_3COOH) was purchased from Cicarelli (Buenos Aires, Argentina). Solutions were prepared with ultra-pure water.

2.2 Modification of Chitosan with glucose by Maillard Reaction

With some modifications, chitosan modification with glucose was performed following the method proposed by Abdelaal *et al*. (Abdelaal, Sobahi, and Al-Shareef 2013). Briefly, 1.5 g of native Chit were dissolved in 100 mL of 1% (V/V) CH_3COOH and then incubated at 35 ºC for 4 h, respectively. The reaction medium was dialyzed with Mw cutoff 12-14 kD membrane (Sigma-Aldrich). G-Chit was spray dried with Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Switzerland).

Static Light Scattering determined the molecular weight (Mw) of G-Chit according to the methodology proposed by (Arata Badano *et al*. 2019) using a Malvern light scattering photometer (Malvern 4700 with goniometer) with a laser at 488 nm. Deacetylation degree (DD) of G-Chit was determined by proton nuclear magnetic resonance (1H-NMR) with a Bruker UltraShield 600 Plus under a static magnetic field of 14.1 T operating at 600 MHz and equipped with an AVANCE-III console. The G-Chit obtained have a degree of deacetylation of 74.7% and an Mw (49 ± 9) 10 kD and a solubility of (0.81 ± 0.02) gL^{-1}, substantially higher than medium Mw Chit (0.22 ± 0.02) gL^{-1}.

2.3 Preparation of composite multilayer films

Previous to preparing the composite multilayer film, the surface of the CPE was activated by applying an anodic potential of 1.200 V for 5 min in Na_2CO_3 saturated solution. The LbL film was prepared by alternating immersions of the CPE in solutions containing G-Chit (0.5 $mg\cdot mL^{-1}$ prepared in deionized water, pH 5) or GOx (2 $mg\cdot mL^{-1}$ in PBS pH 7.0). The adsorption time was 30 min for both polyelectrolytes.

2.4 Electrochemical measurements

Cyclic Voltammetry (CV) and chronoamperometry (CA) studies were carried out with a potentiostat /galvanostat (Teq4, Buenos Aires, Argentina). Experiments were performed in a three-compartment electrochemical cell. A large-area platinum wire and an Ag/AgCl/3 M NaCl electrode (Model RE-5B, BAS) were used as counter and reference electrodes, respectively. The reported potentials are referred to as this reference electrode. All experiments were performed at room temperature. A 0.10 M phosphate buffer solution (PBS) at pH 7.0 was used as a background electrolyte, with 2.0 x 10^{-4} M FcMe as an artificial mediator of the enzymatic reaction. Solutions were deoxygenated by controlled N_2-bubbling for 20 min preceding the measurements, and the gas flow was kept over the solution during the experiments. CA experiments were performed at 0.350 V under convective conditions using controlled magnetic stirring. The current response was registered as a function of time after the sequential addition of glucose aliquots of chosen concentrations.

3. RESULTS AND DISCUSSION:

3.1 Activation of the CPE surfaces and construction of the self-assembled structure

It is known that electrochemical activation techniques can be used to improve the electrochemical characteristics of electrodes based on carbon materials. During electrochemical activation, the amount of a binder at the electrode-solution surface interface decreases, which produces electrochemical signal increments (Chebotarev *et al*., 2018). Furthermore, it has been proposed that activation is accompanied by the elimination of adsorbed impurities, an increase of active sites in the edge plane, and, in some cases, the generation of surface oxides (Motta and Guadalupe 1994). Therefore, CPE was activated in an alkaline medium to optimize the electrochemical response and obtain greater reproducibility. In addition, the electrochemical response of $[Fe(CN)_6]^{3-}$ was used to analyze the effect of the treatment. This is a redox mediator commonly used in the electrochemical characterization of electrode surfaces since its electron transfer kinetics have been extensively studied (Goldstein and Van de Mark 1982).

Figure 1 shows the cyclic voltammograms registered in $[Fe(CN)_6]^{3-}$ before and after the activation procedure. For the unactivated electrode, a peak separation of 0.398 V is observed, which decreases to 0.148 V after activation treatment, demonstrating an improvement in the reversibility of the system. At the same time, a substantial increase in the peak anodic and cathodic currents are observed. These results agree with previous studies (Rice, Galus, and Adams 1983; Chi *et al*. 1997). They have been attributed to the decrease in surface hydrophobicity due to the treatment, which allows the approximation of the ions to the surface of the electrode.

After optimizing the surface treatment, we proceeded to construct the self-assembled structure. Figure 2 shows the chemical structure of the polycation and the self-assembled procedure.

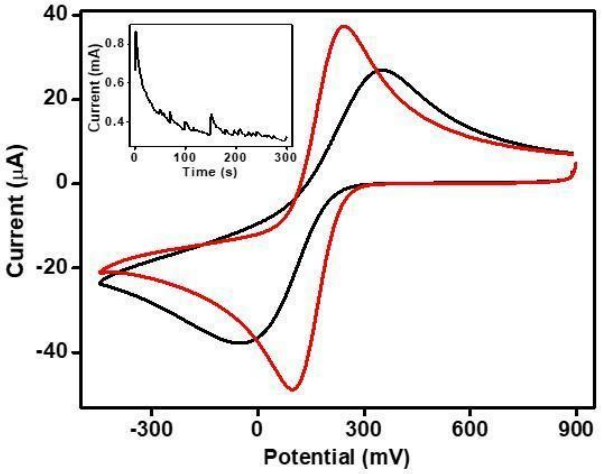

Figure 1. CV at CPE before (black line) and after (red line) activation procedure in 0.10 M PBS pH 7.0 containing 5.0 x 10^{-3} M $[Fe(CN)_6]^{3-}$. Scan rate 100 $mV\cdot s^{-1}$. Inset: the curve of current vs. time corresponding to the activation procedure in Na_2CO_3 saturated solution.

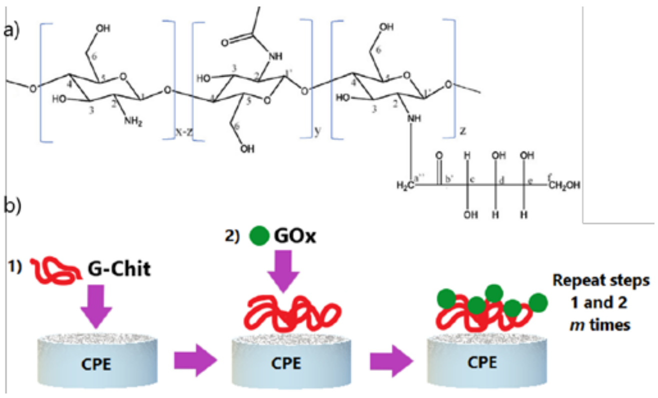

Figure 2. a) G-Chit structure and b) Construction of self-assembled structure at activated carbon paste electrodes.

To avoid their denaturation, the construction of multilayers that include enzymes and proteins preferably requires mild conditions such as neutral pH and room temperature. In this context, the improved water solubility of G-Chit, about Chit, makes it possible to dissolve the former in water, in the required quantity (0.5

mg·mL^{-1}), at a difference of Chit that requires strong acid conditions (pH< pKa ~ 5.0. In an aqueous solution, the polymer has a zeta potential of (89±7) mV that allows the construction of the multilayer structure with GOx negatively charged at the pH of the assembly (the isoelectric point of the enzyme is 4.2).

3.2 Evaluation of the enzymatic response

The enzymatic response at CPE-modified electrodes was analyzed by CV. Figure 3 shows cyclic voltammograms of CPE/(G-Chit/GOx)$_m$, where m corresponds to the number of bilayers (m=1,2,3,4) in an N_2-saturated solution. In the absence of glucose, reversible redox waves of FcMe are observed. After the addition of 0.050 M glucose, a well-defined sigmoidal voltammetric profile is noticed due to the GOx catalytic reaction at the bioelectrode, according to Equations 1 to 3.

$$FcMe_{red} \rightleftharpoons FcMe_{ox} + e \qquad \text{(Eq. 01)}$$

$$GOx(FAD) + G \rightleftharpoons GOx(FADH_2) + GL \qquad \text{(Eq. 02)}$$

$$GOx(FADH_2) + 2FcMe_{ox} \rightarrow GOx(FAD) + 2FcMe_{red} \qquad \text{(Eq. 03)}$$

$$G = Glucose;\ GL = Gluconolactone$$

This result suggested that the enzyme has successfully assembled on the electrode surface and remains active. Moreover, the catalytic current, i_{cat}, increases proportionally with the number of G-Chit/GOx bilayers in the range evaluated up to m=4.

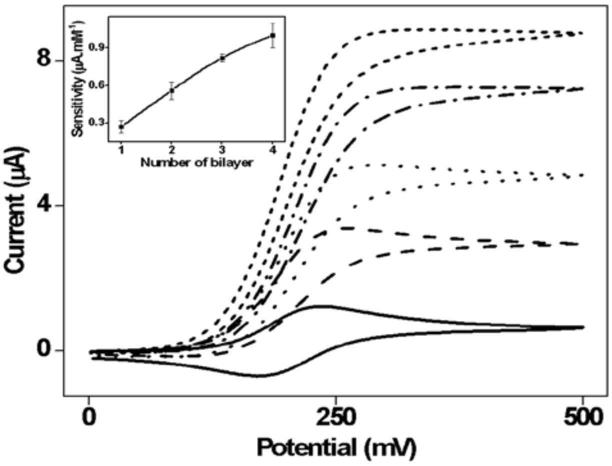

Figure 3. CV at CPE/(G-Chit-GOx)$_m$ in 0.10 M phosphate buffer solution pH 7.0 + 2.0 x 10^{-4} M FcMe in absence (solid line) and presence of 0.050 M of glucose. Scan rate 5 mV·s^{-1}. Inset: Variation of sensitivity as a function of bilayer number.

The influence of enzyme concentration used in the construction of CPE/(G-Chit-GOx)$_3$ was evaluated by analyzing the sensitivity obtained in CA experiments from the fit of the linear range in the calibration plot. The values obtained are (0.44 ± 0.06), (0.82 ± 0.03), and (0.8 ± 0.1) µA·mM^{-1} for 1, 2, and 5 mg·mL^{-1} of the enzyme, respectively. The sensitivity value duplicates when the enzyme concentration increases from 1 to 2 mg mL^{-1} and then remains unchanged, indicating that the maximal amount of enzyme adsorbed is reached under this condition.

The ionization state of the amino acids in the active site can affect enzyme activity (Grahame *et al.*, 2015). Thus, pH has a significant function in maintaining the proper conformation of the active site in the enzyme. The pH effect on the biosensor response was evaluated by CV in the range from 5.0 to 9.0, adding 0.050 M glucose to the cell. The ratio between the i_{cat}, and the current in the absence of glucose, i_b, were calculated. The maximum response, i_{ca}/i_b, was obtained at pH 7.0 (Figure 4); hence, it was chosen as the optimum pH for further studies. The results are an average of five measurements.

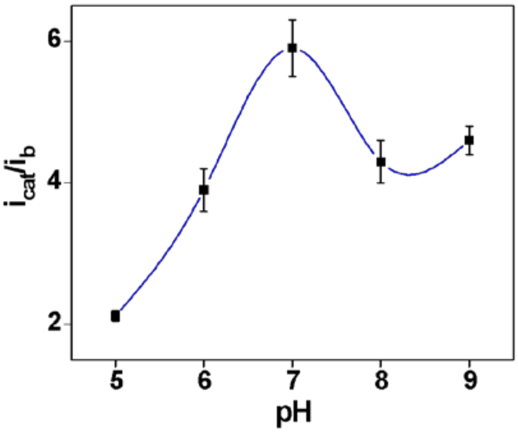

Figure 4. Effect of pH on the response of the CPE/(G-Chit-GOx)$_1$ at 0.486 V in 0.10 M phosphate buffer at different pH + 2 x 10^{-4} M FcMe and 0.050 M of glucose.

The electroanalytical properties of the bioelectrode immediately after preparation for the optimized experimental conditions: m=3, GOx 2 mg·mL$^{-1,}$ and pH 7.0 were analyzed. Figure 5 shows the current-time recorded and the inset corresponding calibration plot. The detection limit (LOD) and LOQ were calculated using the criterion of 3 x SD/s or 10 x SD/s, respectively, where SD is the standard deviation of the background current and *s* the sensitivity (Paz Zanini *et al.*

2016). The linear range of operation is (0.18-1.55) mM, while the values obtained for LOD, LOQ, and sensitivity obtained are 60 µM, 180 µM, and (0.82 ± 0.03) µA·mM^{-1}, respectively.

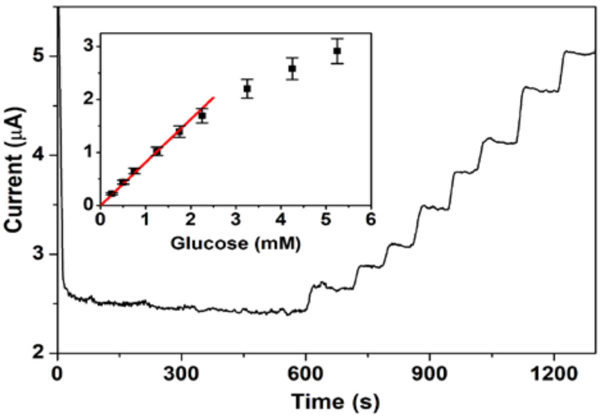

Figure 5. The current vs time plot and calibration curve were obtained at CPE/(G-Chit-GOx)$_3$ at 0.486 V in 0.10 M phosphate buffer plus 2.0 x 10^{-4} M FcMe.

There is little information reported in the literature about multilayer systems built on CPE that include GOx so, it is very difficult to compare the analytical parameters obtained with those of other biosensors of similar composition.

Therefore, and to highlight the analytical characteristics of the developed biosensor, we compare it with others built in CPE, which use enzymatic mediators derived from ferrocene. In this context, we find that the sensitivity obtained is similar to that of a CPE modified with functional GOx/silica/lignin hybrid material, 0.78 µA·mM^{-1} (Jędrzak *et al*. 2018), and significantly higher than that of a Cellulose Acetate/GOx modified CPE, 0.290 µA·mM^{-1} (Sanjaya *et al*. 2021).

The bioelectrode was tested in real samples of commercial milk La Serenisima®. The obtained value for the seven consecutive determinations was (2.2 ± 0.2) g·L^{-1}. According to a t-test at 95% confidence level, these values agree with the manufacturer-reported value, e.g., 2 g·L^{-1}. In addition, to check possible matrix effects, the percentage of relative recovery (RA%) of the current signal produced by an aliquot of 0.25 mmol L-1 glucose before and after adding 10 µL of the sample was measured and calculated by Equation 4.

$$RA(\%) = \frac{i_2}{i_1} x\ 100\% \qquad \text{(Eq. 04)}$$

Where i$_1$ and i$_2$ are the amperometric signals before and after adding the sample. (Burns, Danzer, and Townshend 2003). The sample volume used produced %RA values ranging 62-85%.

4. CONCLUSIONS:

Multilayer films made of G-Chit and GOx were successfully built onto CPE. The best responses were obtained with 2 mg·mL^{-1} of enzyme, 3 bilayers of polyelectrolytes, and working at pH 7.0. Under optimized experimental conditions, the biosensor exhibits a sensitivity of (0.81 ± 0.03) µA mM^{-1} and a linear range of (0.18 to 1.75) mmol·L^{-1}.

Although obtaining high sensitivity values is one of the most desired characteristics in developing a biosensor, it is also important to achieve devices that involve a simple preparation procedure. In our case, we can claim that the developed biosensor combines both characteristics as a consequence of the ease and economy in the construction of a CPE and the versatility of the layer-by-layer assembly.

The analytical response of the biosensor was tested in milk samples with negligible matrix effects. Results show that G-Chit appears promising for the immobilization of enzymes.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the samples analyzed.

5.2. Acknowledgements

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5.4. Competing Interests

The authors declare that they don't have any conflict of interest that exists in this publication.

5.5. Open Access

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Review paper

ADVANCES IN NATURAL EXTRACTS USED FOR ANTIBIOTIC-RESISTANT BACTERIA TREATMENT: THE GRAM-NEGATIVE CASES

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ABSTRACT

Background: Infectious diseases are a global problem, the second human cause of death. Infectious diseases caused by pathogenic bacteria have been treated with a high degree of efficacy. However, even when the 20th century was considered the "golden age" of antibiotics, bacteria developed a different resistance mechanism to antibiotics. In 2017, the WHO issued an alert about 12 bacteria with an urgent need to develop new antibiotics. **Aims**: The aim of the present review is to analyze the current knowledge of the antibacterial activity of natural extract-based treatments against the pathogens listed by WHO. **Methods**: A systematic review of the literature in PubMed was performed to search for publications describing the use of natural extracts as antibiotics over bacteria. We focused on the Gram-Negative group. The exclusion criteria consisted of limiting papers on natural extracts tested over the bacteria culture related to eight selected bacteria, according to an alert issued by WHO in 2017, and seven plant extracts. **Results**: All the Gram-Negative bacteria listed in 2017 by WHO have been treated, with different degrees of advance, with some of the plant extracts and plant-based compounds reviewed. In general, the first approach is using inhibition disks applied over the bacterial biofilm in solid culture media. **Discussion**: While Salmonellae and P. aeruginosa have been extensively studied, over N. gonorrhoeae, A. baumannii have been tested with fewer natural extracts. Edible herbs are more often used, as well as artemisa and wine byproducts. In all cases, they are in the early stages of study, not being tested in patients at present. **Conclusions**: Plant extracts and plant-based compounds are effective as antibacterial, with minimal effects on the host cell. Furthermore, they are sustainable, environmentally friendly, and renewable.

Keywords: *natural extract, bacteria, pathogen, Antibiotic-resistant, infectious diseases*.

1. INTRODUCTION

Currently, infectious diseases are the second leading cause of death in the world. The 20th century was considered the golden age of antibiotics, contributing to diminishing morbidity and mortality caused by infections worldwide. However, the massive use and overuse of antibiotics allowed bacteria to acquire resistance to antibiotics. Antibiotic resistance is the ability of pathogens to avoid the mechanism the drug uses against them. Increasing antibiotic resistance (Diallo *et al.*, 2020) in emerging and reemerging bacterial diseases is a public health issue that must be addressed. Epidemiological surveillance programs work together in different countries to diagnose antimicrobial resistance worldwide (Christaki, Marcou, and Tofarides, 2020; Diallo *et al.*, 2020). In 2017, the WHO alerted and called researchers to find effective treatments against twelve bacteria, eight of which were Gram-negative. An important approach to finding new and effective treatments for infectious diseases is using plant extracts and natural compounds (Mulat, Pandita, and Khan, 2019). In the present review, we focused our attention on recent literature describing the use of natural extracts to develop new antibiotics against bacterial pathogens. The extracts can be the first step in discovering new active biomolecules or even the antibiotic itself. We summarized recent findings of antibacterial activity extracts and bioactive molecules from aromatic plants, wild plants, and plants used as infusions and byproducts of the wine industry.

2. METHODS

A systematic review of the literature in PubMed was performed to search for publications describing the use of natural extracts as antibiotics over bacteria, collecting and analyzing data. In order to do so, we used the following words/terms in combination: bacteria: (name) AND (natural extract) AND (plant name) AND (antibacterial activity or antibiotic). The exclusion criteria consisted of limiting papers on natural extracts tested over the bacteria culture related to the eight Gram-negative bacteria over the eleven present into an alert issued by WHO in 2017 and seven plant extracts. The search was conducted on papers published until September 2021.

3. RESULTS AND DISCUSSION:

3.1 Results

3.1.1 Antibiotic resistance

Antimicrobial resistance is a global public health crisis that complicates our success in treating bacterial infections. After discovering penicillin, Sir Alexander Fleming warned about the potential risk of underdosing antibiotics, which can lead to antimicrobial resistance. In the last decades, the main problem was the opposite, the overdose of antibiotics, not just in humans but also in animals and agriculture (Laxminarayan *et al.*, 2013). The increasing antibiotic resistance (AR) is a major obstacle in infection management. It has been reported in several countries a high and alarming number of human deaths caused by multidrug resistance: 25.000 deaths in Europe in 2009, according to The European Center for Disease Prevention and Control, 23.000 per year in 2013 just in the USA, according to US Centers for Disease Control and Prevention, and finally 10 million deaths per year are estimated globally by 2050 (Diallo *et al.*, 2020). Each antibiotic can exert its effect at different levels, affecting directly or indirectly essential processes for the bacteria, as shown in Figure 1. Antibiotic resistance can be intrinsic, acquired (involving modifications in their DNA by transformation, transduction, or conjugation), or adaptative (through modulation of gene expression) (Christaki *et al.*, 2020). These modifications can be transient or permanent and allow bacteria to survive in the presence of antibiotics by several mechanisms, including the destruction or modification of the antibiotic by the bacteria, modification of the target (as target mutations, replacement, protection, or overproduction), as well as the reduced permeability or increased efflux of the molecule (Christaki *et al.*, 2020). The emergence of multidrug-resistant bacteria or superbugs is followed closely by international surveillance programs. In 2017, an alert was issued about 12 bacteria requiring urgent antibiotic development due to their increased resistance, being *Acinetobacter baumannii, Pseudomonas aeruginosa* (priority 1), *Staphylococcus aureus, Enterococcus faecium, Helicobacter pylori, Campylobacter spp, Salmonellae, Neisseria gonorrhoeae* (priority 2), *Streptococcus pneumoniae, Haemophilus influenzae* and *Shigella spp* (priority 3). The selected bacteria for the present work were Gram-negative bacteria, excluding form the selection *E. faecium*, *S. aureus,* and *S. pneumoniae*

3.1.2 Natural extracts-action mechanisms

The estimated cost to discover and produce a novel antibiotic is 2,5 billion dollars and can take up to 15 years (DiMasi, Grabowski, and Hansen, 2016), which is why the use of natural extracts or natural compounds with antimicrobial activity is a promising approach. The extracts can be obtained from wild plants, cultivated plants, for instance, aromatic and spices, or byproducts of plants and fruits used in the industry. The extract can be used directly as an additive, like some related foodborne pathogens such as *Salmonella*, or it can be used to search in oral or topical administration on the patient. They are also useful for identifying the molecules(s) responsible for biological activity (Mulat *et al.*, 2019). The identification and isolation of bioactive molecules, such as resveratrol, ursolic acid, chlorogenic acid, and several polyphenols, among others, allowed researchers to discover the mechanism of action of those compounds. Using polyphenols as an example, the mechanisms of action for their antibacterial activity have not yet been fully elucidated, but some clues suggest their potential clinical use. It is thought that they can interact with the cell wall, cell membrane, and bacterial proteins, altering processes like bacterial adhesion, metabolite, and ion equilibria, inhibiting biofilm formation, or impairing DNA synthesis (A. Silva *et al.*, 2021). They can also interact with covalently and non-covalently bonding with key bacterial proteins, e.g., penicillin-binding proteins, transporter proteins, surface-adhesion proteins, enzymes (including those involved in DNA synthesis), and cell-wall polypeptides. Additionally, some polyphenols can modulate gene expression (A. Silva *et al.*, 2021). For the present work, we selected extracts from aromatic plants, like rosemary [*Salvia rosmarinus* Spenn., Lamiaceae), syn.: *Rosemarinus officinalis* L.], thyme (*Thymus vulgaris* L., Lamiaceae), and oregano (*Origanum vulgare* L., Lamiaceae), wild plants such as jarilla (*Larrea* spp., Zygophyllaceae) and mugwort or artemisa (*Artemisia vulgaris* L., Asteraceae) and some of the industrial interest as yerba mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) and byproducts of the wine industry from *Vitis vinifera* L. (Vitaceae).

The raw material of the plants can be obtained from several parts, including leaves, tails, roots, the whole plant, the aerial fraction of the plant, and also from the fruits: skin, seeds, or the whole fruit. In the case of *I. paraguariensis*, the commercial presentation ready to use for the preparation of the infusion, called “mate”, is the most commonly used. For *V. vinifera*, we also included wine byproducts and wine as raw material, as resumed in Figure 2. Starting from this material, researchers have been working with extracts, concentrates (only for wine), or Essential Oils (EO).

3.1.3 Bacteria

All the listed bacteria were treated with some of the above-mentioned natural extracts to test their antibacterial activity. It is worth highlighting the great differences in progress: while *Salmonella* and *Pseudomonas* have been extensively investigated, *N. gonorrhoeae*, *Shigella,* and *H. influenzae* have been hardly tested with natural extracts. Differences are also found in the same bacteria, as in the case of *Salmonella*, where not all strains tested with the same extract respond similarly to treatment.

In general, the first approach is the use of inhibition disks. The disk is embedded with the extract or the natural compound in solution and applied over the bacterial biofilm. The inhibition zone is measured concerning the diameter of the area around the disk where the bacteria cannot grow. The solutions can also be added to the liquid or solid culture medium, and growth is monitored by counting the colonies. There are critical parameters to measure when the inhibition is being tested. Minimal Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of the organism. Also, the Minimal Bactericidal Concentration (MBC) provides information on the lowest concentration at or above the MIC required to kill a microorganism. There are also specific parameters, such as MIC_{90}, which indicates the minimum concentration at which 90% of the isolates were inhibited, or IC_{50}, the inhibitory or effective concentration for 50 % of all surveyed isolates of a strain (CLSI, 2020; John E. Bennett, 2020).

Intracellular bacteria need to test their activity once the bacteria invade the host cell. The ideal antibiotic must kill the intracellular bacteria without affecting the cell viability.

The extracts and essential oils tested over each bacteria are summarized in Table 1.

3.1.4 Acinetobacter baumannii

Acinetobacter baumannii is an extracellular, strictly aerobic, nonmotile, Gram-negative coccobacillus. It grows well in conventional culture media. It has been implicated in a wide spectrum of infections, mostly nosocomial. The main anatomical site of colonization is the respiratory tract, which is why it plays an important role in patients in intensive care with mechanical ventilation. Over the past two decades, *A. baumannii* resistance has increased, particularly to beta-lactams, aminoglycosides, fluoroquinolones, and most recently to carbapenems (John E. Bennett, 2020).

Even though *A. baumannii* is at the top of the priority list of WHO, and there is not a significant amount of work on using natural extracts as a specific antibacterial. Among the extracts on which the present review focused, just artemisa was found.

3.1.4.1 Artemisa.

The advances in natural extracts based on artemisa use the aerial part of the plant and/or endophyte of the plant, like *Burkholderia*. The first reports on this class of extracts consist of a phytosynthesis of silver nanoparticles using an extract from the aerial parts of *A. marschalliana* Spreng. When the nanoparticles were used at a concentration of 100 ppm, the inhibition zones were comparable with those reached with an antibiotic, ampicillin, with 11.45 and 15 mm, respectively (Salehi *et al.*, 2016). The role of silver nanoparticles is mainly the interruption of bacterial membranes. Using the pigment extracted from the endophytes *Burkholderia sp*. WYAT7 isolated from the medicinal plant *Artemisia nilagirica* (Clarke) Pamp, Ashitha *et al.* showed inhibition zones of 14 mm, the smaller zones when compared with other bacteria included in the study, like *Salmonella typhi* or *Klebsiella pneumoniae* (Ashitha, Radhakrishnan, and Mathew, 2020). Working with *A. khorassanica* Podl. (syn.: *A. oliveriana* J.Gay ex Besser) extracts, Fatemi, and col. found a high inhibitory activity against isolated strains of *A. baumannii*, with MICs from 6.25 to 12.5 mg/mL for the aqueous extract and 2.23 to 4.46 mg/mL for the methanolic extract (Fatemi, Sharifmoghadam, Bahreini, Khameneh, and Shadifar, 2020). In the study, they also showed the extracts were able to improve the action of two antibiotics, amikacin and imipenem, when they were used in combination, displaying synergism with MICs from two to ten-fold lower.

3.1.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic, extracellular Gram-negative bacterium that grows very well in a wide variety of culture media, even with few requirements. *P. aeruginosa* is related to superficial infections from which it can cause bacteremia. It is one of the most important pathogens concerning hospital-acquired infections, especially pneumonia associated with ventilatory assistance. It also plays an important role in patients with cystic fibrosis. It presents a resistance mechanism associated with beta-lactamases and carbapenemases (John E. Bennett, 2020).

3.1.5.1 Artemisa.

Two species of artemisa have been tested against *P. aeruginosa, and A. mexicana* Willd. ex Spreng*.*, and *A. nilagirica.* In 1996, twelve plants used in Mexican traditional medicine were used to prepare methanolic extracts and confronted four bacterial pathogens. Among the most effective extracts against *P. aeruginosa,* was the extract of *A. mexicana,* with a MIC of 10 mg/mL (Navarro, Villarreal, Rojas, and Lozoya, 1996). In the case of *A. nilagirica,* Ahameethunisa *et al.* used several extraction solvents and tested all of them over the bacteria. When the inhibition zone was determined, the larger zone was 13 mm and the smaller 8 mm, following the decreasing order according to the solvent used to prepare the extract, hexane > methanol = petroleum ether > ethanol = chloroform > diethyl ether. Interestingly, the methanolic extract showed the lower MIC, being of 32 μg/mL, followed by ethanol, hexane, and diethyl ether with 128 μg/mL and chloroform and petroleum ether with 512 μg/mL (Ahameethunisa and Hopper, 2010). The key difference in the main compounds of the methanolic extracts was the presence of amino acids and tannins. In a recent study, the methanolic extract of *A. nilagirica* was used against seven bacterial and one fungal culture. Among them, the most sensitive was *P. aeruginosa*, which also exhibited a dose-dependent response, with inhibition zones of 16, 17, and 19 mm at 100, 150, and 200 μg concentrations, respectively (Parameswari, Devika, and Vijayaraghavan, 2019).

3.1.5.2 Jarilla.

Several works have shown the capacity of jarilla's extracts to inhibit and/or prevent infections with *P. aeruginosa*. Two papers from Micalizzi's laboratory partially purified proteins of crude aqueous extracts of *Larrea divaricata* Cav. The proteins were used for the production of antibodies in mice. On the one hand, they showed a cross-reaction between proteins of *L. divaricata* and proteins of *P. aeruginosa,* specifically cellular and extracellular bacterial proteins. This cross-reaction may indicate antigenic determinants in proteins of jarilla that generate antibodies, and they recognize epitopes that are distributed in proteins of *P. aeruginosa* (C. Sasso *et al.*, 2012)*.* Even more, they also described the capacity of these antibodies to inhibit the hemolytic and proteolytic activity of hemolysins and proteases produced by *P. aeruginosa* (C. V. Sasso *et al.*, 2012)*.* On the other hand, since the antibodies elicited against jarilla proteins of crude extract cross-reacts with cellular and extracellular bacterial proteins of *P. aeruginosa,* Canale *et al.* analyzed the possibility of the cross-reaction with superficial proteins of the bacteria. They found that the antibodies do not just recognize superficial proteins but also opsonize the bacteria, facilitating phagocytosis, which could be crucial for the elimination of the bacteria (Canale, Dávila, Sasso, Pellarín, and Mattar Domínguez, 2018).

3.1.5.3 Oregano.

Several reports have used oregano as EO or extract. For example, Elgayyar *et al.* worked with 12 different commercial EO from herbs and spices used in gastronomy against foodborne pathogens, spoilage bacteria, yeasts, and molds. The most effective was the EO of oregano, with a strong inhibition (zone of 46 mm) over *P. aeruginosa,* and even stronger inhibition against other pathogens such as *Escherichia coli* O:157:H7, *Yersinia enterocolitica* (87 mm) (Elgayyar, Draughon, Golden, and Mount, 2001). In opposition to these results, when Sarac and Ugur prepared their *O. vulgare* subsp. *hirtum* (Link) A.Terracc. EO did not find an inhibitory effect over *P. aeruginosa* even after performing a similar test (inhibition disks) and when they assayed the EO over other Gram-negative bacteria (Sarac and Ugur, 2008). López *et al.* consistently described a weak inhibition with *O. vulgare* EO applied over bacteria and fungus. The Gram-negative bacteria were the most resistant, and *P. aeruginosa* was almost unaffected with concentrations of 6 to 10 % (V/V), showing just a small decrease in colony number with 12% (V/V) of *O. vulgare* EO (López, Sánchez, Batlle, and Nerín, 2007). Interestingly, when a methanolic extract of *O. syriacum* L. was tested, it was the most effective extract to inhibit the growth of *P. aeruginosa,* with a MIC of 2 mg/mL, also showing the lowest MIC for *Candida albicans* and *Staphylococcus aureus* (Assaf, Amro, Mashallah, and Haddadin, 2016). In recent work, researchers found that the antibacterial activity of the oregano extract can be increased by using silver nanoparticles up to 1.3 fold (Meretoudi *et al.*, 2021) when they compared the inhibition zone of the extract versus the extract in silver nanoparticles.

3.1.5.4 Rosemary

Extracts and EO of rosemary have been extensively used as antibacterial. Santoyo and col obtained an EO by supercritical fluid extraction and tested several preparations against bacteria and fungus. The inhibition zones for *P. aeruginosa* were from 19 to 23 mm, similar to the ones obtained for *E. coli*. The MBC was between 1.75 to 2 mg/mL. They also analyzed the composition of the EO and tested the main compounds, being borneol the most effective, with 24 mm of inhibition and an MBC of 1.5 mg/mL (Santoyo *et al.*, 2005). When Sacco *et al.* extracted the non-volatile phenols from rosemary leaves and tested different fractions of the extract, they described a small inhibition (high MBC) for *P. aeruginosa*, with an MBC of 200 μg/mL, in comparison with 130 μg/mL for *S. aureus* (Sacco *et al.*, 2015). Assaf *et al.* also studied *S. rosmarinus* methanolic extracts. They obtained similar results as they showed for *O. syriacum*, MIC of 2 mg/mL, and the combination of both extracts resulted in a MIC of 1+1 mg/mL (Assaf *et al.*, 2016). Comparable results were found with commercial *S. rosmarinus* extract, with a MIC of 6,25 mg/mL and complete biofilm elimination after 5 minutes of incubation with the concentrated extract (200 mg/mL) (J. R. de Oliveira, de Jesus, *et al.*, 2017). In 2018, Gonelimali *et al.* showed that aqueous or ethanolic extracts have no activity against P. aeruginosa, but they were very active against *E. coli* or *S. aureus* (Gonelimali *et al.*, 2018).

3.1.5.5 Thyme

Several *Thymus* species have been tested against *P. aeruginosa* with different effectiveness. EO of *T. kotschyanus* Boiss, and Hohen. and *T. persicus* (Ronniger ex Rech. f.) Jalas were very effective against *S. aureus*, but they did not inhibit the growth of *P. aeruginosa* (Rasooli and Mirmostafa, 2003). However, EO of *T. vulgaris* was the best over eight different EO tested against *P. aeruginosa*, with an inhibition zone of 45 mm and a MIC of 5% (V/V) (N. Silva, Alves, Gonçalves, Amaral, and Poeta, 2013). When Martins *et al.* examined the activity of the extracts with different extraction methods as hydroalcoholic extract, infusion, or decoction, they found a weak halo with the first two methods and a moderate halo with the last one, being in all cases more effective against *E. coli* (Martins *et al.*, 2015). Two interesting works were published in 2017 by Luciane Dias de Oliveira's laboratory. In the first one, they showed the capacity of *T. vulgaris* L. to inhibit the growth of *P. aeruginosa* and prevent biofilm formation (J. R. de Oliveira *et al.*, 2017). The same work described the absence of cytotoxicity and genotoxicity against several cell lines, such as HeLa, MCF-7, and, more importantly, murine macrophages RAW 264.7. In their second publication, they showed that phagocytosis of *P. aeruginosa* by murine macrophages RAW 264.7 was enhanced in the presence of thyme extract, even more than thymol, in contrast to the effect on *S. aureus*, where thymol had higher activity than thyme extract (J. R. de Oliveira, Figueira, *et al.*, 2017). Quite the opposite, Gonelimali *et al.* did not find inhibition of *Pseudomonas*'s growth when they used aqueous or ethanolic extracts of thyme (Gonelimali *et al.*, 2018).

3.1.5.6 Wine byproducts

When Mayer *et al.* (2008) treated *P. aeruginosa* with grape seed extracts, they found a very low activity compared to other bacteria like *H. influenzae* or *S. aureus.* Nevertheless, they observed a fraction of their purification, composed mostly of monomers like catechin and epicatechin, active only against *P. aeruginosa* but not for any other bacterium studied (Mayer *et al.*, 2008). In the same direction, Cueva and col. treated seven different bacteria with seven phenolic wine compounds and six oenological phenolic extracts, with *P. aeruginosa* being one of the less sensitive treatments (Cueva *et al.*, 2012). Purified compounds like gallic acid or ethyl gallate showed the strongest activity, with an IC_{50} of 205 and 289 μg/mL, respectively. The best grape seed extract was an oligomeric-rich fraction (GSE-O, 21 % flavan-3-ols monomers, and 78 % procyanidins), with IC_{50} of 253 μg/mL (Cueva *et al.*, 2012). Using pressed grape pomace from Merlot and Syrah and different extractions methods, strong inhibitions were obtained against *S. aureus*. However, just one of them was effective against *P. aeruginosa*, the extract from Merlot prepared by supercritical fluid extraction, obtained at 60 ºC and 250 bar of pressure, but not the ones obtained at 200 or 300 bar, or 250 bar and 50 ºC (D. A. Oliveira *et al.*, 2013). Wine samples have been tested against bacteria, as in the case of commercial wines of different grape and origin, used in 2017 by Radovanovic *et al.* They assayed fifteen different wines over six Gram-positive and six Gram-negative bacteria, being the Gram-positive more sensitive. Among the Gram-negative*, P. aeruginosa* resulted the second most sensitive, with 3 Cabernet Sauvignon and one Merlot being the strongest inhibitors, with a MIC and a MBC of 125 μg/mL (Radovanović, Arsić, Radovanović, Jovančičević, and Nikolić, 2017).

3.1.6 Helicobacter pylori

Helicobacter pylori is a highly mobile, spiral-shaped, Gram-negative, extracellular bacteria. It shows slow growth and has complex nutritional requirements. It has a tropism for the gastric mucosa, which is why it plays an important role in gastrointestinal diseases in adults and children. Its treatment is complex, using a wide range of antimicrobials. In recent years, the appearance of resistance to clarithromycin, used in several available therapeutic regimens, has been described (John E. Bennett, 2020).

3.1.6.1 Artemisa

In a big screening performed using extracts from 50 Taiwanese folk medicinal plants, artemisa ethanolic extracts prepared with leaves and stems of *A. argyI* H. Lév. and Vaniot were classified as moderate anti-*H. Pylori*-activity herbs, being 9 of 10 *H. pylori* strains inhibited by their herbal extracts (Wang and Huang, 2005). Jeong *et al.* used artemisa extracts *in vivo* to treat *H. pylori* in infected animals. They supplemented the food of mice with the extract and analyzed their tissues and the presence of molecular markers for the diseases. They found that the extract could rejuvenate H. pylori atrophic gastritis and suppress tumors produced by the bacteria (Jeong *et al.*, 2016). From *Artemisia ludoviciana* subsp. *mexicana* (Willd. ex Spreng.) D.D. Keck aqueous extracts, the reported MIC was 250 μg/mL (Palacios-Espinosa *et al.*, 2021). In the same work, the gastroprotective and anti-inflammatory activities were assessed using different solvents using four different fractions from the liquid-liquid extraction process. The fraction obtained with dichloromethane was the most effective, followed by the ethyl acetate fraction. Finally, they identified 2 compounds responsible for the antibacterial activity: estafiatin and eupatilin (Palacios-Espinosa *et al.*, 2021).

3.1.6.2 Rosemary, jarilla, and oregano

The first report on the use of rosemary extracts to inhibit the growth of *H. pylori* in vitro was published in 1996. Among several plant-based extracts assayed, rosemary extracts of 0.4 % (W/V) displayed inhibition zones of 10 and 8 mm when extracted in water or ethanol, respectively (Tabak, Armon, Potasman, and Neeman, 1996). For jarilla (*L. divaricata*) extracts, leaves and tender branches were used to prepare it with four different protocols: cold extract, infusion, decoction, or simulated digestion, and their MICs against 7 strains of *H. pylori* were determined. The measured MIC values depended more on the strain treated than the extraction protocol. The values were between 0.04 to 0.1 mg/L (Stege *et al.*, 2006). Finally, using *O. vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum* EO, Lesjak and col. found a MIC of 2.0 μL/mL for both EO and *Satureja hortensis* L. (Lamiaceae) EO. When they tested binary or ternary combinations of the EO in different proportions, they found synergistic and additive effects, being *S. hortensis: O. vulgare* subsp. *hirtum 2:1 is* the best combination, with a MIC of 0,5 μL/mL (Lesjak *et al.*, 2016).

3.1.6.3 Thyme

In 1996, Tabak and col. used a series of natural extracts to inhibit the growth of *H. pylori*, finding the thyme extract the most powerful (Tabak *et al.*, 1996). The first approach was using inhibition disks, and based on those results, they tested different species of thyme: *T. citriodorus* Schreb., *Coridothymus capitatus* (L.) Rchb. f. and *T. vulgaris*. Among them, *T. vulgaris* showed the strongest inhibition, even stronger than nalidixic acid, cotrimoxazole, sulfamethoxazole, or colistin sulfate. Moreover, when the extract was added to a liquid medium, the bacterial growth was completely inhibited at 3,5 mg/mL (Tabak *et al.*, 1996). Another specie of thyme used was *T. kotschyanus* Boiss. and Hohen., tested against 70 isolated *H. pylori* strains from children 4-15 years old. They found that 66 % of the isolates were sensitive to extracts of *T. kotschyanus* (Farahnaz Nariman, Fereshteh Eftekhar, 2004).

3.1.6.4 Wine byproducts

The extracts are often prepared from different grape varieties, and also, the main isolated bioactive molecule, resveratrol (3,5,4'-trihydroxystilbene), is used. Starting from wine, Mahady and Pendland prepared a wine concentrate by drying and treating the *H. pylori* and adding it to the culture media. They found an MIC_{50} of 50 μg/mL, while the resveratrol solution assayed showed an MIC_{50} of 12,5 μg/mL (Mahady and Pendland, 2000). The same laboratory also prepared 2 new extracts: a Pinot Noir wine concentrate and a second extract by dissolving 1500 ml of red wine in 3 L of methanol, concentrating under reduced pressure, filtering, and collecting both the filtrate (alcohol soluble) and the particulate matter (alcohol insoluble). Only the concentrate and the alcohol insoluble fractions were active, with MIC of 50 and 25 μg/mL, respectively (Gail B. Mahady, Ph.D., Susan L. Pendland, Pharm.D, and Lucas R. Chadwick, 2003). Extracts from whole grapes (variety Colorino, Sangiovese, and Cabernet Sauvignon) were tested against 2 different strains of H. pylori. The determination of the MBC showed the Colorino grapes as the most active, with a MBC of 1.35 and 3.57 mg/mL for the strains G21 and 10K, respectively, after 24 hours of incubation. In the same period, Sangiovese and Cabernet Sauvignon grapes extracts showed activity only against G21 strain, with an MBC of 4 mg/mL (Martini *et al.*, 2009). When they used the main compounds of the extract, resveratrol showed the best activity against both strains, G21 and 10K (MBC 68 and 84 μg/mL, respectively), while myricetin, gallic acid, and quercetin showed MBCs three to four times higher (Martini *et al.*, 2009).

3.1.7 Campylobacter spp

The genus *Campylobacter* comprises small, mobile, extracellular Gram-negative bacilli with a curved morphology. They are aerobic and microaerophilic, with important nutritional requirements for their growth. It is most frequently found to cause gastroenteritis in humans. It is not usually treated, but erythromycin is the most widely used antibiotic when the condition is severe. There are species of *Campylobacter* that are resistant to beta-lactams. With the wide administration of fluoroquinolones, resistance to this group of antimicrobials is observed (John E. Bennett, 2020).

3.1.7.1 Artemisa

In 2011, Castillo *et al.* published the results of a big screening of plant extracts and their activities against *Campylobacter jejuni* and *Campylobacter coli*. (Castillo, Heredia, Contreras, and García, 2011). Their work showed *A. ludoviciana*'s extracts as one of the most powerful against the bacteria. With larger inhibition zones and lower MIC (4-4.5 mm and 0.5-0.6 mg/mL, respectively), they also found a strong reduction of *C. jejuni* and *C. coli* at the cell surface of Vero cells when they pre-incubated the bacteria with the extract of artemisa. Furthermore, their results confirmed a strong inhibition of the activity of the best-characterized *Campylobacter* toxin, the cytolethal-distending toxin (Castillo *et al.*, 2011). Unfortunately, they did not continue the research.

3.1.7.2 Oregano and thyme.

Both extracts have been used comparatively in several works, although the first report used thyme only. Ethanolic extract and post-hydrodistillation residue were assayed against *C. jejuni*, reaching MICs of 1.25 and 0.625 μg/mL, respectively (Šikić Pogačar, Klančnik, Bucar, Langerholc, and Smole Možina, 2016). Both extracts reduced the cell adhesion by up to 35 % compared to the control, and they were more effective when the extract was pre-incubated with the cells, and the bacteria were added later. In comparative research, Ozogul and col. found the oregano and thyme extracts are the most active against several foodborne pathogens, including *C. jejuni* (Ozogul, Kuley, Ucar, and Ozogul, 2015). In both extracts, carvacrol was determined as the main compound, and the authors indicated the molecule responsible for its antibacterial activity. Carvacrol activity has been reported, inhibiting motility without affecting their growth or flagella, and abolishing infection of epithelial cells (van Alphen, Burt, Veenendaal, Bleumink-Pluym, and van Putten, 2012).

3.1.7.3 Rosemary

An interesting difference between the most commonly used methods, diffusion disks and agar dilution, was found while investigating rosemary activity against *C. jejuni*. The researchers used four commercial rosemary extracts, two oil-soluble and two water-soluble extracts. The inhibition zones showed very weak activity for the water-soluble extracts, with a MIC of 40 mg/mL (Klancnik, Guzej, Kolar, Abramovic, and Mozina, 2009). However, when they used the extract in liquid culture media, they found a MIC of 5 mg/mL for the water-soluble extract and 0.625 mg/mL for the oil-soluble extract. The author explained the difference by arguing a potentially weaker diffusion or other antagonistic effects of the tested substances in *Campylobacter* solid medium and, consequently, higher MICs in agar. Interestingly, they also tested two of the most bioactive molecules in the rosemary extracts, carnosic acid and rosmarinic acid, with MICs of 5 and 1.25mg/mL, respectively (Klancnik *et al.*, 2009). In a later work, using rosemary EO, Ozogul and col found a weak inhibition of the rosemary EO against *C. jejuni*, compared with the effect of other 11 EO or the effect of the rosemary EO against other bacteria (Ozogul *et al.*, 2015).

3.1.7.4 Wine byproducts

Castillo and col performed the screening. *Vitis vinifera* grape extracts showed small inhibition zones when applied to the bacterial cultures (Castillo *et al.*, 2011). Nevertheless, later work showed the capacity of grape extracts to inhibit the growth of *C. jejuni*. Using the waste of wine production, grape seed, and skins of Pinot Noir variety, the MIC was 1.25 mg/mL, and when using commercial resveratrol, it was even lower, 0.313 mg/mL (Klančnik *et al.*, 2017). They also described a change in the morphology of the bacteria, from spiral to nonspiral or short bacillary forms. Also, the attachment to cells was diminished by up to 20 % when they treated the cultures with very low concentrations of extract (50 μg/mL).

3.1.8 Neisseria gonorrhoeae

Bacteria of the species *Neisseria gonorrhoeae* have a cocci-like morphology, and are Gram-negative, strictly aerobic, and facultatively intracellular. They have strict nutritional demands for their growth that hinder their growth. It presents tropism for the simple columnar epithelium in the urethra, endocervix, rectum, pharynx, and anus. It is the causal agent of gonorrhea, the second most prevalent sexually transmitted infection in the world. Over the years, *N. gonorrhoeae* has developed a wide variety of resistance mechanisms that have caused a delicate situation due to the few available antimicrobial treatment options based on ceftriaxone and azithromycin (John E. Bennett, 2020).

N. gonorrhoeae is the least tested with natural extracts from the WHO list. Besides some publications with garlic extracts, the only work with extracts we listed uses one of the main compounds found in grape and wine extracts, resveratrol.

3.1.8.1 Wine byproducts.

In 2001, Docherty et al assayed the activity of resveratrol on the growth of six pathogenic bacteria, including *N. gonorrhoeae* and *N. meningitidis*. The results obtained were promising, with an inhibition of 50 % of the growth with 25 mg/L and a total inhibition with 75 mg/L for *N. gonorrhoeae* while for *N. meningitidis* were 100 and 150 mg/L respectively (Docherty, Fu, and Tsai, 2001). Unfortunately, the research group did not pursue the investigation.

3.1.9 Salmonellae

The genus *Salmonella* is made up of intracellular motile Gram-negative bacilli belonging to the family Enterobacteriaceae. They are facultative anaerobes that are easily grown in conventional culture media. They are associated with gastrointestinal conditions and a systemic condition called typhoid fever. In both cases, the interaction of the bacteria with the intestinal mucosa is required. They are also associated with bacteremia and vascular infections. Antimicrobial treatment is usually reserved for typhoid fever, while gastroenteritis requires fluid replacement and symptomatic treatment. The use of fluoroquinolones must be carried out according to the antibiogram results due to the appearance of strains resistant to these antimicrobials (John E. Bennett, 2020).

3.1.9.1 Artemisa

Several species of *Artemisia* have been tested against *Salmonella*, with a high spectrum of results. For example, while *A. argyi* has no antibacterial effect against 17 species of *Salmonella*, while it shows strong activity against *Listeria monocytogenes* (Militello *et al.*, 2011) *A. tournefortiana* EO showed potent antibacterial activity against 20 isolated strains of *S. enteritidis* with a MIC range of 1.95-3.9, 3.9-31.2 and 3.9-31.2 µg/mL, for the hydroalcoholic, aqueous, and hexane extracts respectively (Khosravani, Soltan Dallal, and Norouzi, 2020). In a recent study, Meng-Ting Yang and col. found antibacterial activity against *S. enterica*, working with three variants of *A. argyi* and three variants of *A. indica*

Willd., with MICs from 1.56 to 25 μg/mL

(Yang *et al.*, 2020). They also showed the activity of six main compounds of the EO, with the best results reached when carveol or α-elemene were used. Furthermore, carveol was suggested to play a role in bacterial membrane destruction.

3.1.9.2 oregano

Several *Origanum* species have been used against *Salmonella*, with the dried leaves or the fresh plant, for the extract preparation. In a large screening of EOs from different sources, Hammer and col found MICs of 0.5 and 0.12 % (V/V) for *O. majorana* and *O. vulgare,* respectively (Hammer, Carson, and Riley, 1999). *O. vulgare* ethanolic extract showed similar inhibition zones to gentamicin, with 18 and 19 mm of diameter, respectively, with a MIC of 78.13 μg/mL (Oniga *et al.*, 2018). Obtaining the extracts through hydrodistillation from manually harvested *O. majorana*, Ed-Dra *et al.* found inhibition zones from 15.3 to 18.5 mm working with 8 different *Salmonella* species, with MIC and MBC of 1% (V/V) for all the 8 strains (Ed-Dra *et al.*, 2020). Differences also can be found regarding the culture method of the herbs, as was shown for *O. ehrenbergii* Boiss. wild versus cultivated. The antimicrobial activity of the EOs against *Salmonella enterica* serovar *Typhimurium*, was tested, with the wild herb essential oil showing superior efficacy (Barbour *et al.*, 2014).

3.1.9.3 Thyme and rosemary

These aromatic herbs have been used to prepare EOs and tested against several *Salmonella*'s strains. They were included in the screening of Hammer *et al.*, showing similar MIC, higher than 2 % (V/V) (Hammer *et al.*, 1999). Similar results were shown for rosemary, with a MIC of 2 % (V/V) and moderate activity, with inhibition zones ranging from 8.1 to 10.3 mm (Ed-Dra *et al.*, 2020). When they are used in parallel, there is always higher activity in the thyme EO, independently of the incubation temperatures, as was shown with four different strains of *Salmonella* (Al-Nabulsi *et al.*, 2020), with a difference of 3 times when comparing the inhibition zones. In the case of *T. hirtus* subsp. *algeriensis* Boit. and Reut., was also found to have a moderate difference in their antibacterial activity depending on their growth region in Tunisia, were the herb cultivated in Kairouan (lower altitude, minor thermal amplitude) have the lowest antibacterial activity (Fatma, Mouna, Mondher, and Ahmed, 2014). The authors found a correlation between the strongest activity of the EO and their antioxidant activity, also related to their higher content of monoterpene alcohol.

3.1.9.4 Wine byproducts

Extracts from seeds, skins, and grape pomace have been tested for their capacity for *Salmonella* growth inhibition. Working with grape skin extract obtained from red grapes *Vitis labrusca* L. (Isabel varietal), Aiub *et al.* demonstrated a variable activity over different strains when the extract was pre-incubated with *S. typhimurium* strains TA97, TA98, TA100, and TA102, with a survival up to 32 % using 10 μg/ml over TA102 strain (Aiub *et al.*, 2004). A major concern is the putative genotoxicity that those compounds can induce; in the study, they showed no mutagenic effect over *Salmonella* strains or Balb/c 3T3 fibroblasts (Aiub *et al.*, 2004). Using skin and seed extract, the antibacterial effect over *Salmonella poona* was dose-dependent, being more efficient with the Gram-positive *Bacillus cereus* (Serra *et al.*, 2008). The authors also studied olive extracts, which did not affect *S. poona*. Starting from the same material, seeds, and skins of black grapes, Lluis *et al.* found a strong growth inhibition over *S. typhimurium* strains TA1537 and TA98, with a dose of 0.05 mg. In comparison, a dose of 5 mg was less effective in TA100 (Lluís *et al.*, 2011), highlighting that different strains respond differently to the treatments. The same work showed that a dose of 5000 mg/Kg did not produce an acute toxic effect on rats, and there was no mutagenic effect on mammalian erythrocytes. A recent study used grape pomace extracts to test the IC_{50} over *S. enterica* subsp. *enterica*, with an IC_{50} of 0.752 % (V/V), showing that grape marc was even richer in bioactive compounds than skin extracts compared with previous works. In a comparative study, the seeds extract of three different grape varieties, Hasandede, Emir, and Kalecik Karasi, were tested using agar diffusion disk technique in *S. enteritidis* and *S. typhimurium* cultures. The diameter of the halo of inhibition was higher when 10 % (V/V) was used, showing small differences among the grape varieties, ranging from 21.7 up to 27.7 mm being the most effective Kalecik Karasi for *S. typhimurium* and Emir for *S. enteritidis* (Baydar, Sagdic, Ozkan, and Cetin, 2006). By using Ohmic Heating to prepare grape pomace extracts in concentrations of 1 mg/ml on diffusion disks, a moderate halo formation was found when citric acid or methanol was used in the extraction. However, no halo was generated with water or lactic acid as solvents (Coelho *et al.*, 2021), showing the importance of the solvent used for the extraction.

3.1.9.5 Yerba mate

The extracts of yerba mate are generally prepared from commercial presentations. Gonzalez-Gil and col. reported a high antibacterial activity against *Salmonella* when they added the extract to bacterial suspensions (Gonzalez-Gil *et al.*, 2014). They found a MIC of 7.4 mg/mL, and the extracts were active against *S. enteritidis* 13A, *S. tiphymurium* DT104, and *S. senftenberg*. Once again, the solvent used for the extraction is important in their posterior biological activity, as shown for methanolic and ethanolic extracts over *S. enteritidis* (Martin *et al.*, 2013), where the ethanolic extract produced higher inhibition zones and a lower MIC (3.13 mg/ml). The researchers related the higher activity with the highest total phenolic content obtained with the ethanolic extraction. Similar MICs were obtained from water and acetone:water extracts for different Salmonella strains, ranging from 1.56 to 6.25 mg/mL (El-Sawalhi, Fayad, Porras, Fayad, and Abdel-Massih, 2021).

3.1.10 *Haemophilus influenzae*

Haemophilus influenzae is an extracellular immotile Gram-negative coccobacillus. It is found mainly in the upper respiratory tract. They are aerobic and have some nutritional requirements for their development. It is related to symptoms of otitis media, community-acquired pneumonia, meningitis, exacerbations of chronic obstructive disease, and respiratory infections and can cause invasive conditions such as septicemia. In addition, beta-lactamase-producing strains can complicate treatment with ampicillin and amoxicillin (John E. Bennett, 2020).

3.1.10.1 Artemisa

For treating *H. influenzae*, researchers of China used *A. asiatica* Nakai ex Pamp. EO. Supercritical CO_2 fluid extraction technology was used to extract the essential oil from powdered leaves. The EO proved to have maximum growth inhibition against *H. influenzae*, tested by disc-diffusion method with an inhibition zone of 24.5 mm, MIC of 1.9 mg/ml, and MBC of 4.5 mg/mL, a result close to other antibiotics (e.g., streptomycin and penicillin). Furthermore, examination of the growth curve showed that the EO- treated cultures exhibited a rapid decrease, in contrast to the control, which showed a fast increase (Huang, Qian, Xu, and Huang, 2018).

Another method used to test the bactericidal effect was the leakage of macromolecules (proteins, RNA and DNA) through the cell membrane due to its damage, which was confirmed. Furthermore, the scanning electron microscope observed deformed cell morphology and shrunken cells (Huang *et al.*, 2018).

3.1.10.2 Thyme

Ács and col used six commercial EO, including *T. vulgaris*, against respiratory tract pathogens such as *H. influenzae.* Two methods tested the antibacterial activity of commercial EO: broth macrodilution test to determine MIC and MBC, and vapor phase test to measure MIC produced by the volatile compounds only (Ács *et al.*, 2018). They found a MIC and MBC of 0.11 and 0.22 mg/mL, respectively, for the EO, with a difference in the volatile compounds, where the MIC was 25 μL of EO/L of airspace volume (Ács *et al.*, 2018).

3.1.10.3 Wine byproducts

In 2008, Mayer *et al.* reported the antibacterial activity of grape seed extracts. In their work, extracts from powdered grape seeds were obtained by Aquasolv and by microwave-assisted extraction (MAE). First, the extracts were fractionated on a column, resulting in four fractions (P1-P4). Afterward, fraction P2 yielded fractions S1 and S2 after eluting with different volumes of ethanol.

The raw MAE extracts had some antibacterial activity against *H. influenzae*. Fractions P1+S1 showed moderate activity, while P3+S2 showed high activity. Fraction P3 (250 μg/ml at a dilution of 1:20) proved to be active against *H. influenzae* and other bacterial strains; this fraction contained oligomeric units of catechin and epicatechin (procyanidins). In contrast to fraction P1, which has mostly monomers, and did not prove any activity against them (Mayer *et al.*, 2008).

3.1.11 *Shigella spp.*

Shigella species are nonmotile intracellular Gram-negative rods. They are the causal agent of shigellosis, an invasive condition that affects the colon and rectum, producing epithelial invasion with significant fluid and electrolyte losses. The usual treatment for gastrointestinal symptoms is fluid replacement. In moderate to severe cases, antibiotic therapy can be instituted. High resistance levels to ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolones have been demonstrated (John E. Bennett, 2020).

3.1.11.1 Artemisa

Extracts from two different species of *Artemisia* have been reported as effective growth inhibitors, *A. nilagirica* and *A. dracunculus* L. In the first case, data originated from a large screening using extracts of *A. nilagirica* prepared with different solvents and examined against 11 clinical and 4 phytopathogens causing human illness and damage to major crops. *Shigella flexneri* showed a moderate inhibition zone, from 8 to 10 mm, reaching the larger zones when the extracts were prepared with methanol or chloroform (Ahameethunisa and Hopper, 2010). When the MIC was determined, one of the less sensitive bacteria was *S. flexneri*, with a MIC from 128 to 512 μg/mL. In the second case, extracts of *A. dracunculus* showed inhibition zones over cultures of *Shigella* RSHI only when prepared with methanol but not with chloroform or acetone (Benli, Kaya, and Yigit, 2007). The researchers showed the effect on the bacteria by Electron Microscopy, finding a difference in the morphology of the treated cells, which appeared shrunken with a loss of cytoplasm and depression of the cell walls.

3.1.11.2 Oregano, rosemary, and thyme

Among the three aromatic herbs, oregano is the most investigated so far. In 2003, the three were included, among other 14 species and herbs, for testing against two strains of *S. sonnei* and two strains of *S. flexneri*. In this work, the herbs and species were not extracted but were added directly to the culture media, 1 %(W/V), before their sterilization. The three herbs inhibited the growth of all tested strains except *S. flexneri* CIP. The MIC was determined for all the herbs, 0.5% (W/V). Intriguingly, they found big differences in the inhibition capacity depending on the culture media among MH broth and MH agar (Bagamboula, Uyttendaele, and Debevere, 2003). Using a different species of oregano, *O. libanoticum* Boiss., and preparing the extract with the whole plant, Barbour *et al.* showed strong inhibition of *S. dysenteriae*, reaching 99,9% of inhibition when the disks were loaded with 20 μL of extract. Interestingly, when the disks were loaded with 10 μL of extract, the inhibition was 11.1 % (Barbour *et al.*, 2004). Another specie tested was *O. minutiflorum* O. Schwarz and P.H. Davis; their extracts were prepared with different solvents. In all cases, the extracts showed important antioxidant activity. Regarding the antibacterial activity, it was used in diffusion disks against five different bacteria, obtaining the biggest inhibition zones for *S. sonnei* RSKK 878, independently of the solvent used. When comparing solvents, the descendent order in function of their inhibition zone was *n*-hexane, acetone, ethyl acetate, ethanol, methanol, suggesting the more bioactive molecules are non-polar (Oke and Aslim, 2010). In the same sense, the lowest MIC was achieved with *n*-hexane extracts (125 μg/mL).

3.1.11.3 Wine byproducts

Radovanovic *and col.* inhibited bacterial growth using different crude commercial wines. By using wines of several varietals from Bosnia, Montenegro, Serbia, and Macedonia and adding wine samples to the culture broth, they were able to inhibit the growth of several Gram-negative and Gram-positive bacteria. Nonetheless, *S. sonnei* was the least sensitive among the Gram-negative bacteria (Radovanović *et al.*, 2017).

3.2 Discussion

Bacterial infections can be the origin of diseases with a variety of symptoms and consequences that can lead to death in humans, other animals, and plants. Currently, infectious diseases are the second leading cause of death in the world.

Although the 20th century was considered the golden age of antibiotics and helped to reduce morbidity and mortality caused by infections worldwide, the increasing antibiotic resistance of various pathogenic bacteria has changed the situation and has become necessary for the development of new antibiotics.

Among the strategies to discover new molecules effective against bacteria, using natural extracts is a promising approach to finding bioactive compounds or even an extract or essential oil as a therapeutic agent.

In this review, we have highlighted the latest advances in this field in relation to Gram-negative bacteria, which were included in a 2017 alert from WHO. There is a wide range of results, from wild plants to herbs used in gastronomy to byproducts of the wine industry, including the wine itself.

The classical and conventional methods need to be enriched with new technologies such as nanotechnology, biotechnology, and cellular and molecular biology to improve their ability to penetrate the cell wall, their activity, their targeting, and their delivery.

The natural extracts are active, sustainable, environmentally friendly, and renewable, in line with the latest trends in industrial development. Replacing classical solvents with natural deep eutectic solvents would help to reduce the environmental impact. It will be necessary to continue research, including the mechanism of action, activity at the molecular level, toxicity towards the host cell, and clinical phases in humans.

4. CONCLUSIONS

The emergence of multidrug-resistant bacteria and the emerging and reemerging infectious diseases are important public health problems and big challenges for researchers. Multidisciplinary approaches are necessary to find a solution.

Plant extracts and plant-based compounds are effective as antibacterial, with minimal effects on the host cell. Furthermore, they are sustainable, environmentally friendly, and renewable. They even can increase the added value of industrial processes like wine and infusions elaboration, as well as juice production, using byproducts of the elaboration itself.

The natural extracts and their bioactive compounds can be used alone or in combination with traditional treatments. In all cases, they must be validated *in vitro* and *in vivo* and pass all 5 phases successfully to be used in humans. Most of the reports involved *in vitro* assays and some of them have been tested in eukaryotic cells or mammals to discard adverse effects. They sometimes achieved the patent to be used (Guglielmi, Pontecorvi, & Rotondi, 2020).

Of the bacterial strains alerted by WHO, *N. gonorrhoeae and A. baumannii* are the least tested with natural extracts. On the other side, *Salmonellae*, and *P. aeruginosa* are the most studied. Nevertheless, *P. aeruginosa* been shown to have a very low susceptibility to the natural extracts treatments; even more, there are contradictory results about this issue.

Some foodborne pathogens have been tested not just isolated *in vitro* but also in the food they normally infect, as in the case of *Salmonellae*. In those cases, the extract has an extra requirement, and it must not affect the typical flavors of the food.

Identifying new molecules with antimicrobial activity, improving their purification, and understanding the mechanism of action of natural compounds are essential for finding effective treatments for untreatable infections.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the selected references.

5.2. Acknowledgements

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5.4. Competing Interests

The authors declare that they have no competing interests.

5.5. Open Access

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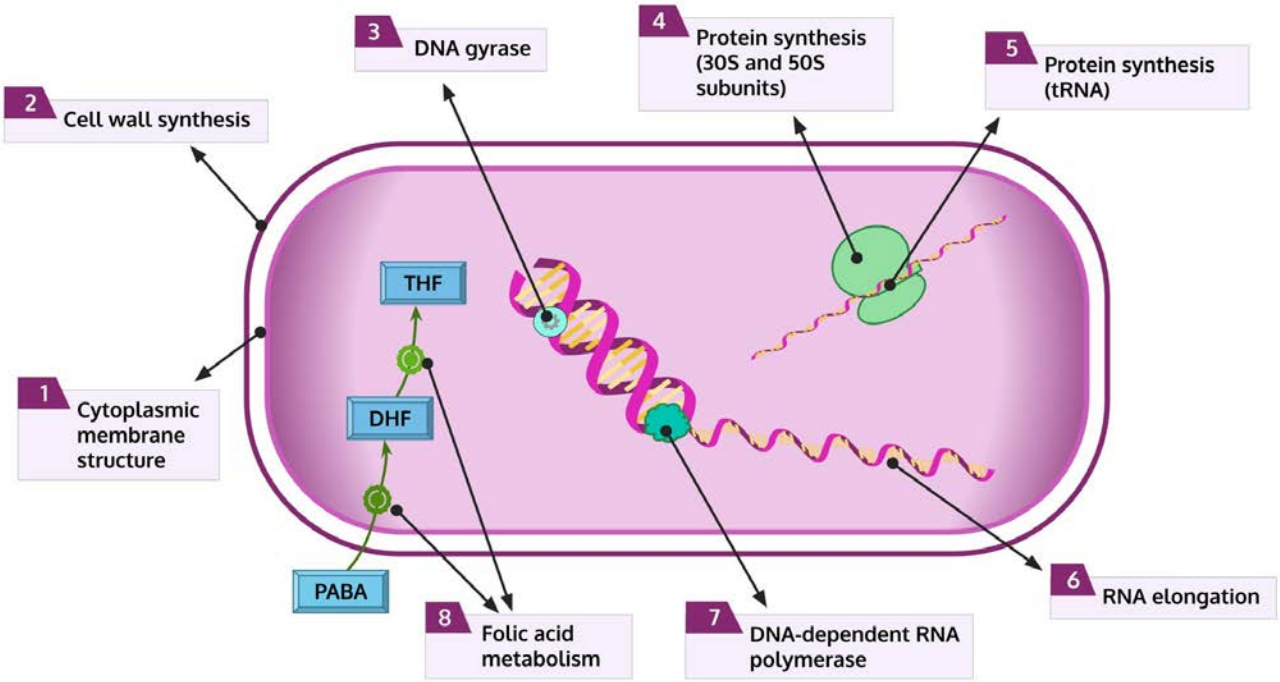
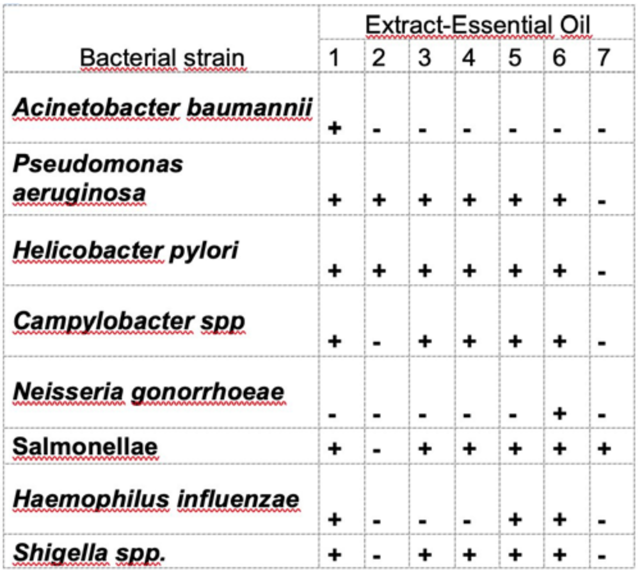


Figure 1. Action mechanisms of antibiotics

1: Changes in permeability or damage to the cytoplasmic membrane. 2: Inhibition of cell wall synthesis. 3: Inhibition of DNA gyrase. 4: Reversible inhibition of protein synthesis by subunit binding (30S or 50S). 5: Inhibition of protein synthesis by prevention of t-RNA binding to the A site. 6: Formation of a stable complex with DNA and RNA elongation prevention. 7: Inhibition of DNA-dependent RNA polymerase. 8: Inhibition of dihydropteroate synthase and dihydropteroate reductase

Figure 2. Raw material for extracts and essential oil preparation. The starting material for the extracts and EOs is, in general, stems, leaves (natural or dried leaves) and for *Vitis vinifera*, seeds, fruit skin, whole fruit, and also byproducts of the wine industry as well as the wine itself.

Table 1. Extracts and EOs used for the bacterial treatment. References: 1: artemisa, 2: jarilla, 3: oregano, 4: rosemary, 5: thyme, 6: wine, 7: yerba mate. +: used, -: not used



Bacterial strain	Extract-Essential Oil						
	1	2	3	4	5	6	7
Acinetobacter baumannii	+	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	+	-
Helicobacter pylori	+	+	+	+	+	+	-
Campylobacter spp	+	-	+	+	+	+	-
Neisseria gonorrhoeae	-	-	-	-	-	+	-
Salmonellae	+	-	+	+	+	+	+
Haemophilus influenzae	+	-	-	-	+	+	-
Shigella spp.	+	-	+	+	+	+	-

SOUTHERN JOURNAL OF SCIENCES

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ASSESSMENT OF THE IRRIGATION WEIR REMOVAL IN THE ENGURI RIVER

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ABSTRACT

Background: Dams are one of the biggest threats to aquatic biodiversity. They restrict the movement of migratory fish. The construction of barriers can cause the complete extinction of some species from the rivers. When a dam can no longer perform its function or research will determine the need to demolish it, dams are often removed. In the lower part of the Enguri River, Georgia, an irrigation weir is currently non-functional. **Aims:** This research aims to prove the need to demolish the dam construction on the 44th km of the Enguri River, as it negatively impacts biodiversity and creates an artificial barrier in the river. **Methods:** Visual inspection was used as a method to assess the morphology and habitat of the dam where it is located. The conversation method was used with Engurhesi LTD representatives to understand the current function of the dam. The Questions were related to the current function of the dam. **Results:** Based on the conservation with Engurhesi LTD representatives, the study has shown that there is no reason that an irrigation weir might be left in its current state on the Enguri River. Based on the studies, the damage to biodiversity is real and disturbing. **Discussion:** The irrigation weir on the Enguri River was left untended because of the construction of the Enguri dam. Currently, there is no reason to divert the river Enguri with the help of an irrigation weir as there is no excess water in this river. **Conclusions:** In conclusion, it can be said that it is necessary to remove the irrigation weir on the Enguri River to restore habitat and mitigate the threats to biodiversity.

Keywords: *Dam removal, biodiversity, river restoration, fish passage.*

1. INTRODUCTION

Rivers are susceptible to anthropogenic modification. To fulfill the higher need for freshwater, barriers are constructed for power generation, irrigation, and flood control (Bredenhand and Samways, 2009). However, dams are one of the biggest threats to aquatic biodiversity. They restrict the movement of migratory fish in different parts of rivers (Liu *et al.*, 2019). For some species, it is necessary to move to the upper reaches of the river in order to leave offspring. Dams are insurmountable barriers for such species (Mary *et al.*, 1996). Such migratory species are: sturgeons and salmons (Ferguson *et al*., 2011). The construction of barriers can cause the complete extinction of some species from the rivers (Zhang *et al.*, 2011). Further, dams impede sediment movement downstream of the river. This affects the formation of the seashore. Which, in turn, is associated with high financial costs (Croitoru *et al.*, 2020).

When a dam can no longer perform its function or research will determine the need to demolish it, dams are often removed. Dam removal is a world-renowned practice. More than 60 dams are demolished annually in the United States (Magilligan *et al.*, 2016). For example, to restore the sole stock of Atlantic salmon, France demolished two big dams in the Loire Valley in 1998 (Bednarek, 2001). For a dam to be demolished, it is necessary to assess its economic benefits and environmental impact. In addition, it is necessary to investigate all the consequences that will follow the collapse of the dam (Bednarek, 2001).

In the lower part of the Enguri River, Georgia, 44 km from the Black Sea and 0.7 km from the Enguri bridge in village Rukhi, there is an irrigation weir of 4-5 m in height that is currently non-functional. The weir was built to provide the nearby area with irrigation water in the 50's of the XX century.

This research aims to prove the need to demolish the dam construction on the 44th km of the Enguri River because there is no need for its existence, and it has a negative impact on biodiversity as it creates an artificial barrier in the river.

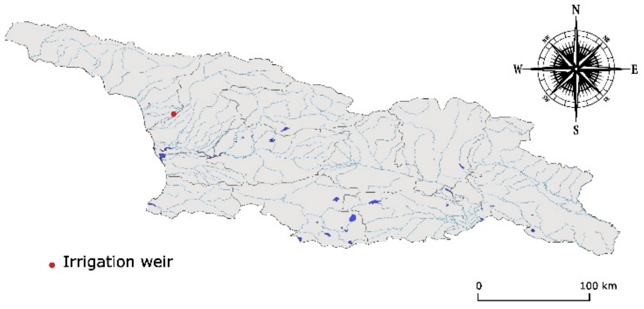
2. MATERIALS AND METHODS

2.1. Study Area

The Weir is located at the following coordinates: 42˚34ˈ33.92ˈˈN; 41˚50ˈ57.85ˈˈE on the Enguri River close to the de facto border of Abkhazia (as Abkhazia is a conflict region, there are security concerns for detailed studies). The elevation is 111 m.

The Enguri river is located in western Georgia. It originates in the high Caucasus, is 213 km long, has a watershed area of 4060 km^2, runs through the mountain valleys to the de facto border of Abkhazia, and emptyes into the Black Sea near the village Anaklia.

The Enguri dam is located on the Enguri River (km 84) in Tsalenjikha municipality, Georgia. It is a concrete arch dam with a height of 271.5 m.



Figures 1 and 2. Maps of an Irrigation wire on the Enguri river, Georgia.

2.2. Methods

Visual inspection was used as a method to assess the morphology and habitat of the dam where it is located. The conversation method was used with Engurhesi LTD representatives to understand the current function of the dam. The Questions were related to the current function of the dam. The questions were: When was the irrigation dam built? What was the purpose of the irrigation wire? Does it now function as it should? Why is it not functioning as it was designed to do?

The location of the dam was added to a Google map. In addition, the distance to the nearest upstream barrier and the length of the liberated part of the river was measured after removing the dam.

3. RESULTS AND DISCUSSION

3.1. Results

Based on the conservation with Engurhesi LTD representatives, the study has shown that there is no reason that an irrigation weir might be left in its current state on the Enguri River. Based on the studies, the damage to biodiversity is real and disturbing.

3.2. Discussions

The irrigation weir on the Enguri River was left untended because of the construction of the Enguri dam. After the construction of the Enguri dam, more than 90% of the water of the Enguri River is relocated to the Gali reservoir with a 15 km pressure tunnel. From the Gali reservoir, the water flows into the Black Sea by the Eristskali River. Moreover, after the conflict in Abkhazia in the 90s, the infrastructure was completely out of order. The Enguri river has historically been a sturgeon river. There were several species of sturgeon to breed up to 70th km (Arnold, 1896; Marti, 1939; Tikhi, 1929). After the Enguri HPP (1961-1978) construction, which is located north of the town Jvari in Tsalenjikha, Georgia, this river lost its spawning function for sturgeons. From the Enguri dam to the irrigation weir, the Enguri River mainly holds the water from the Rivers Magana (10.3 m^3/sec) and Oroli (3.71 m^3/sec). There is a low water period in summer, and irrigation is necessary for this season when there is the least amount of water in the rover. Currently, there is no reason to divert the river Enguri with the help of an irrigation weir as there is no excess water in this river. Prior to the construction of the Enguri dam, the river had the amount of water during flood that needed to be regulated. The research takes the initiative to demolish this weir so it is no longer a barrier to fish migration. Suppose the dam cannot be dismantled for political reasons (the dam is located at the administrative border with the occupied region of Abkhazia). In that case, it will be necessary to arrange a fish pass to allow fish to migrate from the downstream to the upstream. In case of demolition, it will impact the nearby tributaries of the Enguri River, and further studies will be needed.

Figure 3. Irrigation wire on the Enguri river, Georgia.

4. CONCLUSIONS

In conclusion, we can say that there is a necessity to remove the irrigation weir on the Enguri River to restore habitat and mitigate the threats to biodiversity.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the Enguri river and the dam conditions.

5.2. Acknowledgements

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5.3. Funding source

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5.4. Competing Interests

There is no conflict of interest in this publication.

5.5. Open Access

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Review paper

THE TREND TOWARDS PHENOME-WIDE ASSOCIATION STUDIES (PheWASs) IN COVID-19 RESEARCH

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ABSTRACT

Background: Coronavirus Disease-2019 (COVID-19) appears in individuals asymptomatically and in various symptomatic forms. Symptomatic diversity can result in diagnosis failures, hospitalization, admission to intensive care, multi-organ failure, and death. The causes and risk factors of the severity of disease symptoms are uncertain. This uncertainty can only be resolved by elucidating the effects of host genes and genetic variations on different phenotypes. **Aim:** This review aimed to emphasize the importance of large-scale genotype-phenotype correlation studies in elucidating the phenotypic diversity in COVID-19 disease. **Methods:** All publications related to Phenome-Wide Association Study (PheWAS) in the PubMed database were searched. PheWAS studies applied to COVID-19 patients have been identified. In addition, studies applied to the genome-wide association study (GWAS)- Electronic health records (EHRs) data and additionally matched to the gene expression data were systematically reviewed. The latest PheWAS methodology and its importance in Large-scale genotype-phenotype correlations are discussed within the context of published COVID-19 studies. **Results**: According to our PubMed search data, there are few PheWAS studies on COVID-19 disease. This review explains the use of PheWAS studies applied to health records and GWAS data, and colocalization studies applied to expression quantitative trait locus (eQTL) analysis to understand the phenotypic variability of COVID-19. **Discussion**; Although there is a very limited number of PheWAS studies on COVID-19 diseases, these studies have obtained important data. At the current stage, there is a need for such studies in COVID-19 research. **Conclusions:** PheWAS is an ideal method for large-scale genotype-phenotype correlation studies that can reveal genetic diversity and phenotypic diversity in the pathophysiology of the disease.

Keywords: *Phenome-wide association study (PheWAS), Genome-wide association study (GWAS), Electronic health records (EHRs), Large-scale genotype-phenotype correlation, COVID-19.*

1. INTRODUCTION

The new coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) (WHO, 2022). According to the World Health Organization (WHO), data published as of 25 April 2022 shows 507,501,771 individuals have been affected by COVID-19, of which 6,220,390 individuals have died as a result of the disease (Dashboard, 2022). It has caused a global pandemic and constitutes a public health problem (Mahase, 2020). COVID-19 disease can be seen as symptomatic and asymptomatic. The course and severity of the disease are different from person to person (Guan *et al*., 2020; Kotsev *et al*., 2021).

An important question is the role of host genes in COVID-19 infection. To answer this question, we need to understand the effects of host genes and genetic variations on the different phenotypes of COVID-19. Large-scale genotype-phenotype correlation studies have been demonstrated to do this in several different studies *(Gaziano et al., 2021; Moon et al*., 2021; Verma *et al*., 2021). These studies are most beneficial in the presence of clinical heterogeneity. Clinical heterogeneity investigation is needed when different disease phenotypes are compared between case and control groups. Therefore, the differences in endotype, endophenotype, and symptomatic severity of a disease can be understood. Genomic medicine is committed to elucidating the genetic diversity underlying the phenotypic difference in disease (Manolio *et al*., 2013). Genomic medicine differs from traditional genetics in that it regards the functions-interactions of all variations and genes in the genome (Guttmacher *et al*., 2002). This field deals with the hereditary components of monogenic, polygenic, and infectious diseases. Doing this provides an understanding of the molecular basis of all diseases and enables the development of targeted therapy and personalized treatment strategies. Genomic medicine combines multidisciplinary fields and focuses on diseases by matching genetic characteristics with phenotypic data (Manolio *et al*., 2013; Wei *et al*., 2017). The use of genomic medicine strategies has become essential in COVID-19 research. The basis of these strategies involves genome-wide association study (GWAS) data and Electronic health records (EHRs) containing phenotypic data (Linder *et al*., 2021). EHRs include all individual disease, clinical, and treatment information rather than cohorts specific to a single disease (Linder *et al*., 2021). At the same time, since it is comprised of data from large populations, there is a wealth of data covering a variety of population characteristics (Linder *et al*., 2021).

Phenome-wide association studies (PheWASs) have been used in the evaluation of GWAS results (Denny *et al*., 2010). PheWAS determines the full phenotypic spectrum associated with each genetic trait (Hebbring, 2014). PheWAS was initially developed to reveal the large-scale genotype-phenotype correlations of complex diseases. However, it can be used to evaluate the risk score of genetic variations with disease using EHRs *(Denny et al*., 2016). PheWAS has emerged as a popular high-throughput framework mechanism capable of combining EHR data with GWAS data (Linder *et al*., 2021). In addition, data obtained in PheWAS can be matched with tissue-specific expression Quantitative Trait Locus (eQTL) data to help understand the biological mechanism underlying the genotype-phenotype relationship (Moon *et al*., 2021).

This review describes the PheWAS trend from GWAS, and the methodology of PheWAS. Then, genotype and phenotype data resources for COVID-19 research are mentioned. Next, the importance of PheWAS in cross-genotypic-phenotypic correlation and large-scale genotype-phenotype correlation is discussed. Finally, PheWAS studies on COVID-19 patients using GWAS data and EHR-based data were mentioned. This review aims to give an overview of the current state of COVID-19 research, focusing on genotype-phenotype-related studies. PheWAS is an ideal method for large-scale genotype-phenotype correlation studies. As a result, the genetic diversity in pathophysiology and phenotypic variety of the disease can be revealed.

2. METHODS

The method explains the genetic and phenotypic data used in PheWAS analyses. The methodology of GWAS analysis, which is used as a genetic data source in PheWAS, and its integration into PheWAS analysis are detailed. EHRs, which are also included as the phenotypic data set in the PheWAS analysis, were also examined. It is mentioned how the PheWAS analysis is applied to these data. Finally, the phenotypic diversity in covid-19 patients is mentioned.

PubMed / National Center for Biotechnology Information (https://pubmed.ncbi.nlm.nih.gov/) was used as the source of information about the PheWAS analyzes applied in our review. PheWAS research articles on COVID-19 disease were selected, and their results were examined. PheWAS research articles and review articles applied to other diseases were excluded.

In addition, 2 terms (A / B) were considered in selected research articles; A) PheWAS analysis articles containing genotypic and phenotypic information B) articles matching phewas analysis with gene expression data.

The research was conducted from February 01 to July 01, 2022. The period searched on the databases was from 2007 to 2022 (includes the date PheWAS analyzes were first performed and the date to date).

3. RESULTS AND DISCUSSION

3.1. Results

To date, all publications related to PheWAS have been scanned in PubMed (Figure 1). These publications selected articles to establish a large-scale genotype-phenotype relationship in COVID-19 patients. Articles were determined according to A and B terms.

Table 1. *Data from PheWAS publications scanned in PubMed and articles included and excluded from our systematic review*

Term	Database	Results	Exclusions
"A"	PubMed	3	777
"B"	PubMed	3	777

Few studies of PheWAS have been conducted on COVID-19 diseases. However, quite comprehensive and important data have been obtained. Increasing the number of these studies is very important. These studies appear to provide full phenotypic data for each GWAS significant variation or genetic locus (Table 2). We have systematically examined and discussed the COVID-19 phenotypes and comorbidities with which these genetic traits are highly correlated.

3.1.1. The GWAS era and the trend toward PheWAS

Investigating the effects of variations that underlie human genetic diversity has been the focus of attention since the completion of the human genome project. Advancing molecular genetic techniques allow for pleiotropic effect studies in disease research. Pleiotropy is when any variation in the genome affects multiple phenotypes (Tyler *et al*., 2016). Pleiotropy investigates the causes of phenotypic differences in diseases from individual to individual and forms the basis of knowledge for personalized medicine applications (Sivakumaran *et al*., 2011). GWAS has been used for large-scale genotypic-phenotypic data sets to illuminate human pleiotropy *(Hindorff et al., 2009; Sivakumaran et al*., 2011). GWAS investigates the pleiotropic effect at the level of variation between a large number of cases and controls. GWAS simultaneously analysis millions of variations across the whole genome (Consortium, 2007; Hindorff *et al*., 2009). GWASs analyze single nucleotide polymorphisms (SNPs) and can display effects as minor as $P<5\times10^{-7}$ (Consortium, 2007). In GWAS, both chip-based microarray and next-generation sequencing techniques are used for analysis. GWASs usually focus on analyzing variations found in the intergenic, intronic, and exonic regions of the human genome (Li *et al*., 2008). Significant variants found are not always causal; however, linkage disequilibrium (LD) can assist in identifying closely correlated variants (Anderson *et al*., 2011).

Population cohorts were defined through the genetic diversity of different ethnic groups (Benjamin *et al*., 2007). Consortium studies were conducted by combining these population cohorts (Consortium, 2007). These consortium studies have contributed to revealing even minor effect variations and genetic differences in populations (Consortium, 2007). The genetic effect size is defined by looking at the allelic frequency of the variations in cases and controls (Bush *et al*., 2012). According to this genetic effect size, the disease-related genetic risk score of the variation is identified (Consortium, 2007; Hindorff *et al*., 2009).

Furthermore, it can explain the importance of ethnic origin, genetic differences, and genetic predispositions in the pathophysiology of the disease. GWAS has also successfully identified genetic risk factors involved in the epidemiology, development, severity, clinical differences, and response to treatment of the disease (Michailidou *et al*., 2015). Thus, a large number of new genetic traits associated with diseases have been detected (Hindorff *et al*., 2009).

GWAS offers researchers a unique opportunity to demonstrate the effect of variations on disease phenotypes. With the rapid increase in GWAS studies, and the data size increasing from Array-based technology to NGS, there was a need to establish a biobank for these samples and for the ease of access for researchers. Existing GWAS data were brought together by the US

National Human Genome Research Institute (NHGRI) in 2008, and the GWAS catalog was created. In 2010, the GWAS catalog website was established in collaboration with the European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/gwas/). This catalog of associations is increasing every year. The catalog details all current SNP-trait relationships to date from common to rare diseases. The data collected in the catalog is combined with other sources to allow statistical project-centric modeling (Buniello *et al*., 2019).

The GWAS era has accelerated human pleiotropy research and led to cross-phenotype associations studies (Tyler *et al*., 2016). The cross-phenotype association approach has attracted great attention in the scientific world (Denny *et al*., 2010). The first PheWAS was performed in 2010 and was utilized to understand genetic pleiotropy in humans (Denny *et al*., 2010). PheWAS can be considered as an inverse method to GWAS. In GWAS studies, the relationship of many genetic traits with a specific phenotype is investigated. However, in PheWAS analyzes, the relationship of a single genetic trait with many clinical phenotypes is studied (Hebbring, 2014). Therefore, PheWAS analysis is complementary to GWAS in disease research (Hebbring, 2014).

PheWAS enables the simultaneous identification of associations between a genetic trait and phenotypic traits, clinical manifestations, and many diseases. At the same time, genetic predispositions underlying disease comorbidities may also emerge (Karaca *et al*., 2020). Furthermore, it can reveal new genotype-phenotypic correlations as thousands of phenotypes can be compared with significant variations of GWAS (Cronin *et al*., 2014; Denny *et al*., 2010; Karaca *et al*., 2020). For example, one study applied PheWAS to FTO gene variants previously reported to be associated with type 2 diabetes and obesity. They used the eMERGE Network (Gottesman *et al*., 2013) and BioVU DNA biobank data (Roden *et al*., 2008). The study found that the FTO gene variant, associated with body mass index (BMI), is also associated with sleep apnea. Furthermore, the variant associated with obesity, non-alcoholic liver disease, fibrocystic breast disease, and gram-positive bacterial association were defined (Cronin *et al*., 2014).

The web tools GRASP (Leslie *et al*., 2014), GeneATLAS (Canela-Xandri *et al*., 2018), and PhenoScanner (Staley *et al*., 2016) are used to perform PheWAS analysis. These websites contain thousands of GWAS study information with millions of SNP-trait information. It allows users to query the full phenotypic spectrum of each SNP with PheWAS analysis.

The genetic component of PheWAS is not restricted by GWAS significant SNPs. Rare variations (MAF < 0.05), mitochondrial variations, copy number variation (CNV), and structural variation (SV) data can also be analyzed (Basile *et al*., 2016; Mitchell *et al*., 2014). On the other hand, apart from genetic data, clinical analysis results, biochemical parameters, environmental measures, and quantitative values can be used in biomarker studies for disease (Liao *et al*., 2017).

3.1.2. Phenome information collection in PheWAS

PheWAS can be used for matching metadata collected from longitudinal studies *(Denny et al*., 2010; Denny *et al*., 2016). Longitudinal studies identify risk factors for a particular disease over multiple time points. It is a research strategy that includes reproducible observations of the same variables for short or long periods (Shadish, 2002). This strategy also categorizes the personal characteristics of the records that exist retrospectively over time or new data to be collected prospectively. The EHR, which contains demographic and clinical characteristics, is longitudinal in nature (Denny *et al*., 2016). For PheWAS, EHRs are utilized as a source of phenome information (Verma *et al*., 2021; Zhou *et al*., 2021).

3.1.3. EHR-based PheWAS

Electronic health records (EHR) with more than 50 years of history have gained popularity in recent years (Gottesman *et al*., 2013; McDonald *et al*., 1977). Many countries have started to organize their data by establishing national health record systems (Linder *et al*., 2021). EHRs have rapid and automated clinical data collection from when patients are recruited. It contains the individual and social characteristics of diseases. EHRs describe the prevalence, course, and outcomes of diseases at the national level while also providing an opportunity to compare and match across international EHRs (Linder *et al*., 2021). EHR data provides the opportunity for electronic phenotyping (e-phenotyping). It represents a more comprehensive e-phenotyping information collection as they automate clinical data collection (Linder *et al*., 2021). The e-phenotyping information collection produces computational large-scale phenotypic big data in terms of disease monitoring, prevention, and development of preventive health strategies and treatment strategies. As EHR data has accumulated over the years, researchers have highly developed and structured the content of data types. These data types include all observable characteristics of an individual, such as age, gender, BMI, past-existing diseases (hereditary disease, chronic complex disease, infectious disease), drugs used, and allergic conditions (Casey *et al*., 2016; Denny *et al*., 2013; Linder *et al*., 2021).

A procedural medical code system is also implemented within EHRs. This code system called the International Statistical Classification of Diseases, and Related Health Problems (ICD), is a globally accepted system in which medical diagnoses are standardized (Krawczyk *et al*., 2020). The ICD compares and contrasts disease statistics on a global scale. It also procures convenience in identifying the prevalence of diseases, treatment strategies, and taking preventive measures at the international level (Harrison *et al*., 2021). In addition, disease-based phenotypic data algorithms have been developed, including ICD data in the EHR such as PheWAS.

The emergence of EHR and the creation of EHR-linked biobanks allow large-scale genotype-phenotype correlations to be established (Salvatore *et al*., 2021). There are many EHR-linked biobanks, and these biobanks contain multi-omics data as well as genomic data. For example, the UK Biobank (Allen *et al*., 2014), Chinese Kadoorie Biobank (Chen *et al*., 2011), Vanderbilt BioVU (Roden *et al*., 2008), Electronic Medical Records and Genomic Network (eMERGE) (Gottesman *et al*., 2013), which contains data on more than 200 000 individuals worldwide, are among the biobank data sources that can be analyzed in PheWAS. The advancement of machine learning algorithms and the targeting of big data analysis led to the design and development of PheWAS (Gagliano Taliun *et al*., 2020; Salvatore *et al*., 2021). This advancement enables comprehensive correlation analysis based on multivariate regression analysis by integrating multiple datasets (Bush *et al*., 2012). Since PheWAS is a complex big data analysis, it can illuminate the hidden and unknown gene-phenotype relationships associated with any disease (Gagliano *et al*., 2020; Karaca *et al*., 2020; Salvatore *et al*., 2021). It can also scan for the reflection of genetic predispositions leading to population stratification and population-specific disease phenotypes (Bush *et al*., 2012). In addition, combining and comparing different populations data can expose the effect of ancestral-ethnic origin differences on disease phenotypes (Verma *et al*., 2021).

3.1.4. Mapping eQTL information to PheWAS

When PheWAS with phenotype data is applied to GWAS significant variations, the full phenotypic spectrum associated with the SNP is identified. Tissue-specific expression quantitative trait locus (eQTL) data and PheWAS data colocalization analysis can be applied to elucidate the underlying pathophysiological condition in the reflection of genotype to phenotype (Moon *et al*., 2021). eQTL information is available on the Genotype-Tissue Expression (GTEx) portal and is open to researchers (www.gtexportal.org). The GTEx v8 dataset includes whole-genome sequencing (WGS) and RNA-sequencing (RNA-seq) information of 17,382 samples from 838 donors. Gene expression data of cis and trans variations of 52 tissues and two cell lines are also included (www.gtexportal.org). The cis-eQTL value gives the change in expression level relative to the transcriptional start size of genes located close to the LD of the variations (G. Consortium, 2020). In addition, cis-eQTL values can be calculated in multi-tissue and single-tissue (G. Consortium, 2020). In disease studies, the cis-eQTL value in the tissue associated with the disease allows for the interpretation of the variation affect on pathogenesis (G. Consortium, 2020).

3.1.5. Phenotypic diversity in COVID-19 patients

Coronavirus Disease-2019 (COVID-19) is observed in different individuals as asymptomatic and in various symptomatic forms. This diversity can give rise to diagnosis, outpatient treatment, hospitalization, intensive care unit admission, multiple organ failure, and death (Guan *et al*., 2020). The symptomatic variability in COVID-19 is connected with the level of inflammatory response triggered by immune system activation. A systematic immune reaction occurs due to the effector cells involved in the immune response, the release of mediators that mediate inflammation, and their complex interactions. This immune reaction sometimes causes immune hyperactivation or dysregulation, resulting in an abnormal cytokine storm. This uncontrolled immune response results in the development of acute respiratory distress syndrome (ARDS), increased disease severity, multiple organ failure, and even death in COVID-19 infection (Kotsev *et al*., 2021). The basis of this uncontrolled immune response and infection susceptibility, which varies from person to person, is very likely to be based on host genetic diversity.

The causes and risk factors influencing the severity of disease symptoms (mild, moderate, and severe complications) are uncertain. Many studies have been conducted on the severity and mortality rate of COVID-19 disease. In these studies, it has been determined that advanced age, male gender, socioeconomic level, type-2 diabetes, cardiovascular diseases, hypertension, kidney diseases, cancer, obesity, and asthma are connected with severe complications in COVID-19 (Fang *et al*., 2020; Williamson *et al*., 2020). However, these findings are analyzed based on observational and numerical data from limited, regional, hospital-based studies (Williamson *et al*., 2020). It does not reflect the influence of human genotypic structure, genetic predisposition, cross-genotypic-phenotypic correlation, and the importance of population diversity. COVID-19 is not well understood and how host genetic factors contribute to the pathogenesis of disease severity difference and its interaction with its comorbidities (Williamson *et al*., 2020).

3.2. Discussions

Identifying the risk factors that cause COVID-19 severity and symptomatic variation may provide clinical and therapeutic advantages and contribute to developing protective-preventive strategies. Various host genetic traits may likely be risk factors influencing viral susceptibility, immune response, disease progression, and outcomes (Choudhary *et al*., 2021; Debnath *et al*., 2020). GWAS offers the opportunity to identify potential candidate genes associated with severity, development, and symptomatic differences of COVID-19 infection. Since COVID-19 first appeared, numerous GWAS have been performed to identify potential candidate host genetic traits. Variations of *SLC6A20, LZFTL1, CCR9, CXCR6, XCR1, FYCO1* (3p21.31) (Group, 2020), *ABO* (9q34.2) (Wu *et al*., 2020), *HLA* (6p21.33) (Novelli *et al*., 2020), *TMEM189-UBE2V1* (20q13.13) (Wang *et al*., 2020), *ACE2* (Xp22.2) (Hou *et al*., 2020), *TMPRSS2* (21q22.3) (Anastassopoulou *et al*., 2020; Hou *et al*., 2020), *TLR7* (Xp22.2) (Anastassopoulou *et al*., 2020), *ApoE* (19q13.32) (Kuo *et al*., 2020), *IFITM3* (11p15.5) (Thevarajan *et al*., 2020; Zhang *et al*., 2020), *CTSB, CTSL* (8p23.1, 9q21.33) (Lee *et al*., 2020; Yang *et al*., 2021), *PIEZO* (16q24.3) (Cheng *et al*., 2020), *OAS1, OAS2, OAS3* (12q24.13) (Pairo-Castineira *et al*., 2021), *TYK2* (19p13.2) (Pairo-Castineira *et al*., 2021), *DPP9* (19p13.3) (Pairo-Castineira *et al*., 2021), *IFNAR2* (21q22.1) (Pairo-Castineira *et al*., 2021) genes were found to be associated with COVID-19 infection. So far, a large amount of SNP-trait information has been stored in the GWAS catalog (https://www.ebi.ac.uk/gwas/) and at the COVID-19 Host Genetics Initiative (https://www.covid19hg.org/) that can be used in COVID-19 research. However, GWAS has some limitations; i) It can only associate variants with a single phenotype and does not reflect the full phenotypic spectrum *(Kotsev et al*., 2021), and ii) It is insufficient to determine the comorbidities of COVID-19 (Kotsev *et al*., 2021). PheWAS analysis overcomes these limitations by utilizing GWAS data with EHR Data.

Many PheWAS studies have been conducted on COVID-19, matching GWAS and EHR data (Crespi, 2020; Gaziano *et al*., 2021; Lopera *et al*., 2020; Moon *et al*., 2021; Verma *et al*., 2021; Zhou *et al*., 2021) (Table 1). Variations related to COVID-19 severity and severe clinical symptoms in a PheWAS study were associated with many different phenotypes (Verma *et al*., 2021). In the related study: *ABO* locus rs495828 variant was associated with 53 different phenotypes, the most significant of which was detected with venous embolism (Verma *et al*., 2021). The *ABO* locus rs505922 variant was linked with 59 phenotypes and strongly associated with thrombosis count (Verma *et al*., 2021). It has been demonstrated that the *MUC5B* locus rs35705950 variant increases the risk of idiopathic fibrosing alveolitis and is associated with 11 different respiratory features (Verma *et al*., 2021). There was a negative correlation between the risk of CRHR1 gene rs61667602 variant pulmonary fibrosis and *TYK2* locus rs11085727 variant autoimmune conditions (Verma *et al*., 2021). In another study, it was determined that the rs13050728 variant is a risk factor for COVID-19 hospitalization. Through the eQTL analysis, the expression levels of *TPSG1* and *VEGFR2* genes in plasma were lower than in other tissues (Gaziano *et al*., 2021). In addition, the rs4830976 variant was related to COVID-19 hospitalization and caused changes in the expressions of the *ACE2, CA5B, CLTRN, and VEGFD* genes related closely through LD in the eQTL coagulation analysis (Gaziano *et al*., 2021).

PheWAS can be of benefit to elucidating the association of a single disease-related significant locus with intermediate phenotypes (Zhou *et al*., 2021). It is a strategy that can be especially effective for drug and biomarker research (Crespi, 2020). The role of the relevant locus in the pathophysiology of the disease can be revealed using simultaneous eQTL information with PheWASs. For example, to explain the relationship between the 3p21.31 genetic locus and COVID-19 severity, the expression level changes of the genes in this locus caused by the rs67959919 variation were examined (Zhou *et al*., 2021). This PheWAS and eQTL analysis observed that the rs67959919 variation caused changes in the monocyte count by increasing *CCR1* gene expression and decreasing *CCR2* gene expression. Furthermore, it has been revealed that the same variation plays a role in the level changes in eosinophil and neutrophil counts by decreasing the gene expression of the *CCR3* gene (Zhou *et al*., 2021). In another study, seven different GWAS significant variations (rs657152, rs11385942, rs150892504, rs138763430, rs117665206, rs147149459, and rs151256885) with COVID-19 mortality rates were elucidated concerning drugs (Amlodipine and aspirin) and other clinical phenotypes for medical drug targeting (Crespi, 2020). A locus-targeted PheWAS analysis focused on angiotensin-converting enzyme 2 (*ACE2*) and serine protease *TMPRSS2* genes, which are known to be involved in the virus infecting human cells in COVID-19 infection (Lopera Maya *et al*., 2020). During infection, the ACE2 receptor protein is responsible for cell invasion, while the TMPRSS2 protein takes part in preparing the S protein (Yan *et al*., 2020). Genotype-phenotype correlation analysis was performed between 1273 genetic variations (*ACE2* and *TMPRSS2* genes were in and near regions localized) and 178 quantitative phenotypes in the related study (Lopera Maya *et al*., 2020). In the *ACE2* gene, the variant rs17264937 was highly correlated with Eosinophils, and the rs5980163 variant with triglycerides. rs150965978 is associated with plasma levels of CHIT1 protein, while the variant rs28401567 has been reported to be significantly associated with thrombocytes in the *TMPRSS2* gene (Lopera *et al*., 2020).

According to clinical data, individuals with severe clinical diagnosis in COVID-19 also have a pre-existing disease (Fang *et al*., 2020). The ability of PheWAS to define the cross genotype-phenotype correlation is important in the identification of disease comorbidities and in stating the underlying genetic traits. One study focused on determining the comorbid disease-related phenotypes of 22 variations associated with severe COVID-19 respiratory failure and applied PheWAS and eQTL colocalization analysis to GWAS (Moon *et al*., 2021). Five variations (rs647800, rs11385492, rs12610495, rs3934992, rs134130) were significantly associated with 13 different endocrine, metabolic and immunological phenotypes (Moon *et al*., 2021). Variations of rs647800 and rs11385492 have been reported to be risk factors for monocyte-induced inflammation related to the number of monocytes and the percentage of monocytes found in the blood. The rs647800 variation was associated with thrombin time, and they suggested that it may be effective in developing coagulopathies (comorbidity of severe COVID-19). They revealed that the rs12610495 variation is also associated with fibrotic idiopathic interstitial pneumonias (comorbidity of severe COVID-19). The rs3934992 variation was found to be related to the waist-hip ratio (adjusted for BMI), and it was thought to be a risk factor in obesity (comorbidity of severe COVID-19) (Moon *et al*., 2021).

Through PheWAS analysis, changes in risk factors were also observed according to COVID-19 severity and ethnicity differences. In addition, a correlation was observed between the *LMNA* gene rs581342 variation and neutropenia, *HL-DRA* gene rs9268576 variation, and thyrotoxicosis in parallel with the severity of COVID-19 in Africans (Verma *et al*., 2021).

In some COVID-19 studies, PheWAS was applied only to EHR data (Oetjens *et al*., 2020; Salvatore *et al*., 2021; Song *et al*., 2021). In these studies, COVID-19 positive diagnosis, severity, hospitalization, and mortality were matched with the ICD code information of the individuals. Thousands of phenotypic codes (demographic features, clinical findings, biochemical parameters, age, gender, and all genetic and chronic disease information of individuals) were used in these cohorts consisting of thousands of individuals (Oetjens *et al*., 2020; Salvatore *et al*., 2021; Song *et al*., 2021). In addition, some studies focus on ethnic differences (Salvatore *et al*., 2021). For example, in non-Hispanic Whites, Hematopoietic conditions were associated with ICU admission/death, and mental disorders were associated with death (Salvatore *et al*., 2021). Also, in non-Hispanic Blacks, Circulatory system and genitourinary conditions were associated with ICU admission/death (Salvatore *et al*., 2021). As a result, large-scale phenotypic correlations were detected. However, these studies are based on numerical and observational data. Since they were not matched with genotypic data, the genetic predisposition underlying the detected correlations could not be fully understood.

GWAS provides an excellent source of genotypic data. It allows the comparison of sick-healthy individuals and enables the determination of genetic predispositions according to population differences. However, it is not sufficient on its own as it reflects the genetic spectrum associated with a single phenotype. Therefore, there is a need for bioinformatics methods to match phenotypic data with GWAS data. COVID-19 has different symptomatic forms and variations in disease severity from person to person. Clarifying the genetic predispositions underlying these phenotypic diversity is important for elucidating the pathogenesis of the disease and for a good prognosis. As a source of phenotypic data, EHRs provide large-scale data of enormous quality. However, phenotypic data alone are not sufficient to elucidate the disease pathogenesis and prognosis. PheWAS analysis has been developed as a bioinformatics method that can be applied to both GWAS data and EHR data. It can also illuminate the full phenotypic spectrum by matching both data. On the other hand, by matching eQTL data with PheWAS data, it contributes to the elucidation of the molecular mechanisms involved in the pathogenesis. As a result of processing these data together with PheWAS analysis, it provides the opportunity to understand the reasons for the phenotypic diversity of COVID-19.

4. CONCLUSIONS

Performing large-scale genotype-phenotype correlation studies are important to determine an individuals risk for the prevention and evaluation of disease severity and symptomatic variations. In addition, conducting studies on different populations and comparing them on a global scale would also be valuable in elucidating genetic predispositions arising from ethnic differences. At this point, PheWASs emerge as an important approach for profiling large-scale genotype-phenotype correlations. The data already available will prepare the foundation for identifying high-risk COVID-19 individuals, developing protective-preventive treatment strategies, and personalized medicine over the standardized-for-everyone approach. This review provides an overview of the PheWAS methodology and its application to COVID-19 studies. We mention the current methodology, data and analytical resources, and COVID-19-associated genetic variant summaries for future PheWASs. To date, many studies have been carried out globally, and a wealth of data is available from GWASs and EHRs. PheWAS is an ideal approach for large-scale genotype-phenotype correlation studies. The quality, number, and impact of such studies will increase in the near future and gain the importance it deserves. Thus, the role of host genetic predispositions and genetic diversity in phenotypic differences of Covid-19 can be revealed.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the consulted bibliography and the period when the research was conducted (from 2007 to 2022). However, studies on COVID-19 are limited to the years 2019-2022.

5.2. Acknowledgements

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5.3. Funding source

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5.4. Competing Interests

The authors declare no conflict of interest.

5.5. Open Access

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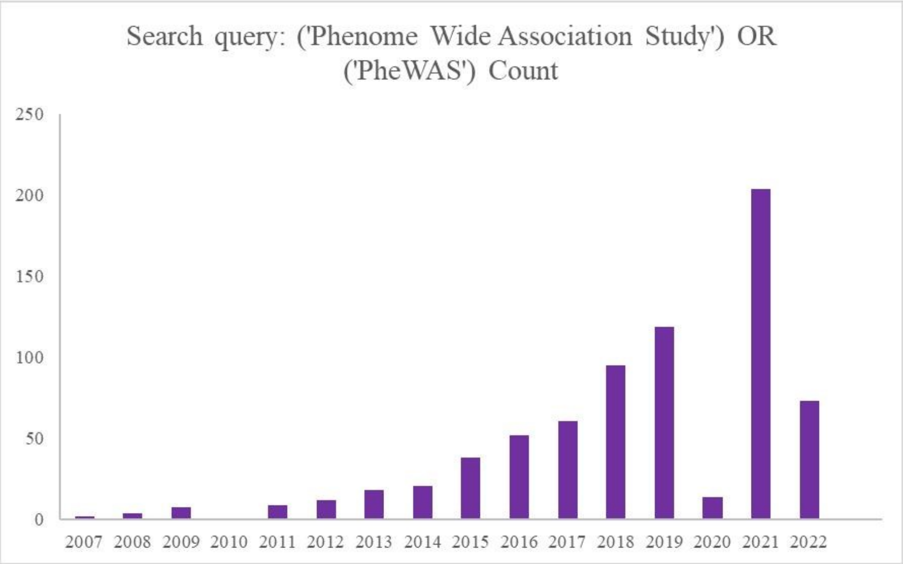

Figure 1. *There are 780 PheWAS publications published in Pubmed so far*

Table 2. *The data of PheWAS analyses carried out to determine the large-scale genotype-phenotype correlation using GWAS and EHR data in COVID-19 research are given in the table. In the PheWAS analyzes performed, there are those with the most significant genotype-phenotype correlation*

Term	**rs number**	**Lokus/Gene**	**Other PheWAS phenotypes**	**Covid-19 phenotypes and severity**	**References**
A	rs495828	*ABO* lokus	Venous embolism	Critical illness and hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs505922	*ABO* lokus	Thrombosis	Critical illness and hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs35705950	*MUC5B* locus	İdiopathic fibrosing alveolitis, respiratory features	Critical illness and hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs61667602	*CRHR1*	reduced risk of pulmonary fibrosis, Post-inflammatory pulmonary fibrosis, Idiopathic fibrosing alveolitis, Other alveolar and parietoalveolar pneumonopathy	Hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs11085727	*TYK2* locus	Psoriasis, Cutaneous lupus erythematosus, Lupus (localized and systemic), Psoriatic arthropathy, reduced risk for autoimmune conditions,	Critical illness and hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs9501257	*HLA-DPB1*	Rosacea	Critical illness of Covid-19	(Verma *et al*., 2021)
A	rs9268576	*HLA-DRA*	Rheumatoid arthritis and other inflammatory polyarthropathies	Hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs111837807	*CCHCR1*	Sarcoidosis, Vitiligo	Critical illness and hospitalization of Covid-19	(Verma *et al*., 2021)
A	rs9896243	*NSF*	Post-inflammatory pulmonary fibrosis	Critical illness of Covid-19	(Verma *et al*., 2021)
B	rs13050728	*TPSG, VEGFR2*		Covid-19 of hospitalization	(Gaziano *et al*., 2021)
B	rs4830976	*ACE2, CA5B, CLTRN, VEGFD*		Covid-19 of hospitalization	(Gaziano *et al*., 2021)
B	rs67959919	*CCR, CCR2*	Monocytes count	Severe Covid-19	(Zhou *et al*., 2021)
B	rs67959919	*CCR3*	Eosinophil count, neutrophil count	Severe Covid-19	(Zhou et al., 2021)
A	rs657152	*ABO* blood group	Clotting time, (PEF) peak expiratory flow, HB concentration, monocyte count, Amlodipine, and aspirin	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs11385942	*LZTFL1, CCR9*	Monocyte, neutrophil, granulocyte, eosinophil and macrophage traits, lymphocyte count, antithrombotic agents, hypertension, Type 2 diabetes, blood clot, DVT (deep vein thrombosis), allergic and atopic diseases, and BMI	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs150892504	*EVAP2*	Platelet count and BMI,	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs138763430	*BRF2*	Lymphocyte count and FEV1/FVC ratio (forced expiratory volume/forced vital capacity), Amlodipine	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs117665206	*TMEM181*	FEV1, PEF, Amlodipine	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs147149459	*ALOXE3*	FVC, PEF, and FEV1	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs151256885	*ALOXE3* (intronic)	Blood clot, eosinophil percentage, DVT, allergic and atopic diseases, Amlodipine and aspirin	Covid-19 mortality	(Crespi *et al*., 2020)
A	rs17264937	*ACE2*	Eosinophils	Covid-19 infection	(Lopera *et al*., 2020)
A	rs5980163	*ACE2*	Triglycerides	Covid-19 infection	(Lopera *et al*., 2020)
A	rs150965978	*TMPRSS2*	Plasma levels of CHIT1 protein	Covid-19 infection	(Lopera *et al*., 2020)
A	rs28401567	*TMPRSS2*	Thrombocytes	Covid-19 infection	(Lopera *et al*., 2020)
B	rs647800		Thyroid-stimulating hormone, Hematocrit, Monocyte count, Hemoglobin concentration, Red blot cell count, Activated partial thromboplastin time, Total kolestrol, Legs-leg fat ratio (male),	severe COVID-19 with respiratory failure	(Moon *et al*., 2021)
B	rs11385942		Monocyte percentage of White cells, Monocyte count	severe COVID-19 with respiratory failure	(Moon *et al*., 2021)
B	rs3934992		Waist-hip ratio (adjucted for BMI)	severe COVID-19 with respiratory failure	(Moon *et al*., 2021)
B	rs134130		Celebellar vermal lobules VI VII	severe COVID-19 with respiratory failure	(Moon *et al*., 2021)
B	rs12610495	*DPP9*	Fibrotic idiopathic interstitial pnemonias	severe COVID-19 with respiratory failure	(Moon *et al*., 2021)

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MANAGEMENT MODEL FOR CONSTRUCTION WASTE PRODUCED IN THE CITY OF RIO DE JANEIRO

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ABSTRACT

Background: A new management model for construction and demolition waste (CDW) was proposed for the city of Rio de Janeiro, hence waste the city planning area estimated generation; the results were compared with the data published by COMLURB and SNIS through the statistics tests was proved that CDW had been dispersed, which support the hypothesis that the current model cause dispersion of the rubble. **Aim:** This study proposes a new management model for CDW to the city, according to regions where occur the higher production of rubble. **Methods:** The estimation of rubble was obtained through the issued licenses from 2006 to 2020 published by SMPU. The quantification of rubble in the city was carried out by adopting the generation indicator from licensed construction activities. The amount of CDW published by COMLURB and SNIS was compared through statistics tests ANOVA and T-Test, the second period from 2006 to 2020 and 2011 to 2020. To measure the area to implant a CDW recycling plant, criteria from production capacity were used. **Results**: ANOVA test to data from 2006 to 2020 and 2011 to 2020, according to a confidence interval of 95%, found the P value 0.589 and 0.022, respectively, it was verified that the significant difference is between data from COMLURB and Estimated. The T-test was applied from the same period in data from SNIS and estimated the P values 0.399 and 0.014. **Discussion:** The data from Estimating between 2006 and 2020 showed the best representation. The *Área de Planejamento – AP* (Planning Area) 4 was where 50.70% of the total rubble, after the AP 3 with 17.66%, the AP 5 was estimated at 16.59%, last of all, AP 2 and AP 1 were calculated the generation of 8.28% and 6.77%, respectively. Therefore, sizing the CDW recycling producing 361,99.00 tons per year demands an area of 32,397.50 m². **Conclusions:** It was concluded that the receipt of CDW in transfer stations managed by COMLURB is not allowed; therefore, the need to create a construction waste recycling plant was presented, to this end, it is necessary to have an available area of approximately 32,397.50 m².

Keywords: *construction waste, recycling, quantification*, *waste transfer stations, landfill*

1. INTRODUCTION

The high generation of construction and demolition (C&D) waste, associated with the unregulated action of agents, implies the imposition of a significant number of degraded areas on the population in the form of clandestine disposals or irregular disposals. The first arises mainly from the actions of companies dedicated to transporting waste from big constructions that unload in usually inappropriate places and without an environmental license. As for irregular deposits, usually in large numbers, they mostly originate from small works or rehabilitation carried out by the urban population most lacking in resources, often through self-construction processes, as they do not have the financial resources to hire regularized companies that operate in the sector, strongly collaborates for the environmental degradation resulting from irregular dispositions due to the performance of small collection vehicles with low displacement capacity (Pinto & González, 2005).

CDW causes worldwide problems; equally important, the estimated generation of this waste in Brazil is higher than 70 Mt/year, around 500 kg/year per capita, thus representing the largest fraction of municipal solid waste. In recent years, there have been advances in policies concerning responsibility in the disposal and recycling of waste in general; however, currently, only a fraction of CDW is recovered (Contreas, Teixeira *et al*., 2016).

In Rio de Janeiro, three waste disposal centers were operating simultaneously: Gramacho landfill, CTR – Gericinó, and CTR – Rio in Seropédica. Equally important to remember that the Jardim Gramacho Metropolitan landfill was deactivated in June 2012, and CTR – Gericinó, the receipt of urban solid waste, was finished in April 2014 (Rio de Janeiro (Cidade), 2021).

The waste disposal center CTR – Rio, in Seropédica, was inaugurated on April 20, 2011, when it started operating in an area of 220 hectares (Rio de Janeiro (Cidade), 2021).

In its first phase, around nine thousand tons of waste were deposited daily, which initially received rubbish from the Jacarepaguá transfer station, where the garbage truck that serves the neighborhoods of Barra da Tijuca, Recreio, and Jacarepaguá are unloaded (Rio de Janeiro (Cidade), 2011).

When it comes to waste management in the city of Rio de Janeiro, C&D, waste generators of construction waste, which produce more than 2m^3 per week, are classified as large generators (Rio de Janeiro (Cidade), 2006).

The collecting and disposing of C&D waste from small generators is the responsibility of the city hall through the Companhia Municipal de Limpeza Urbana - COMLURB (Municipal Urban Cleaning Company). However, for those that exceed this volume, the disposal of such waste becomes the duty of the generator itself, which must hire an accredited company in that institution to dispose of these materials.

COMLURB-accredited companies must comply with requirements related to the accreditation standard concerning the destination of removed waste, such as the § 1° of Art. 17. from Portaria “N” COMLURB N°002, which determines the obligation to dispose of waste from large generators only in places that are part of the urban cleaning system of the city of Rio de Janeiro operated or formally authorized by this corporation (Rio de Janeiro (Cidade), 2022).

Due to the reuse potential of these materials, as well as the high generation, this study consists of developing the estimated generation of construction waste, through formal constructions in the city of Rio de Janeiro, during the period from 2006 to 2020; these values will be compared with the totals of C&D waste published by COMLURB and the Sistema Nacional de Informação Sobre Saneamento - SNIS (National Sanitation Information System). After the analysis, the mapping of authorized site waste disposal in the city will be carried out. The adaptation of waste transfer stations will be proposed to enable the temporary receipt of C&D; the adaptation of those places will follow a priority criterion according to the demand of the regions with the highest generation.

The severity of C&D waste generation does not only depend on the quantity produced but also on its management. In general, the amount and composition of this waste vary between regions, depending on other factors specific to each location, thus influencing its generation, such as population, constructive activity, materials used in construction, and traditions, among others (Villoria Sáez, Porras Amores, & Río Merino, 2020).

As most of the material that we discard and need to be disposed of is not dangerous, it is simply garbage or refuse. Most of the simple constituents of this solid waste are construction and demolition rubble, almost all of which is also reused or eventually buried in the ground. The second largest volume of waste is produced by commercial and industrial sectors, followed by domestic waste from households (Baird & Cann, 2011).

The C&D waste is generated by the loss of materials at construction sites and as a by-product of demolition processes. They are quite heterogeneous, formed by mortar, sand, soil, ceramics, concrete, wood, metals, paper, stone, asphalt, paint, plaster, plastic, rubber, and putrescible materials. The proportion between the materials varies depending on the technology adopted in the construction, as well as the amount produced depends on the greater or lesser heating of the economy in the country (Calijuri & Cunha, 2013).

Anyone who builds or performs makeovers in concrete structures uses cement. Therefore, even if indirectly knowing the cement user, it is possible to establish a profile of the rubble generator (Vilhena, 2018).

As long as cities increase and modernize, there is a growing generation of waste from construction and demolition, whose volume is 30 to 50% of the total urban solid waste produced. They are called C&D waste, which requires specific management due to their characteristics and environmental problems caused by their uncontrolled disposition. A relatively small part of the C&D waste is recyclables, such as plastics, paper, cardboard, wood and others. However, most of it consists of rubble, which can be processed and reused as aggregates in the production of concrete and mortar (Eigenheer & Ferreira, 2011).

The C&D waste generation index is extremely useful for estimating the amount of rubble from a given region or country. However, the values may differ due to the variety of materials used in construction, production, the level of development of the construction industry, and the built environment. According to the analysis of the work, though with a relatively high population density in the city of Shanghai in China, values of waste generation of the order of 724 kg per capita^{-1} year^{-1} and 842 kg per capita^{-1} year^{-1} were recorded in the years 2000 and 2010, respectively. The analysis also indicated that more than 80% of the components of the C&D are composed of concrete, bricks, and blocks, and these materials can be reused if appropriate recycling technologies are applied (Ding & Xiao, 2014).

The index adopted in Spain to estimate the generation of CDW as a function of the built area is used by the Instituto de Tecnología de la Construcción de Cataluña (Institut of Technological Construction in Cataluña), Colegios Profesionales de Arquitectos Técnicos (Professional Associations of Architects' Technicians) and the statistics of the Ministerio de Fomento (Ministry of Public Works), use as a parameter: 120.00 kg/m² for the construction of new buildings, 338.70 kg/m² for rehabilitation, 1,129.00 kg/m² for total demolition and 903.20 kg/m² for partial demolition (Llatas, 2013).

In Brazil, there was an increase in the amount of CDW collected by municipalities between 2010 and 2019, from more than 33 million tons, in 2010, to more than 44.5 million, in 2019. Thus, the amount collected per capita grew from 174.3 kg to 213.5 kg per capita^{-1} year^{-1} (Abrelpe, 2020).

Private construction companies are responsible for the high generation of CDW; for this reason, they have an obligation to develop specific management projects, for example, contemplating screening at construction sites, including the use of registered transporters and licensed areas for handling and recycling. On the other hand, the public authorities must provide an environmentally correct destination for small generators responsible for reforms and self-constructions and cannot implement self-management. In this study, data related to the municipality of Novo Horizonte in the state of São Paulo were presented. The average per capita generation of CDW was around 367 kg per capita^{-1} year^{-1}. As the average composition of the CDW was analyzed predominantly those classified as Class A, representing 91% of the total mass, and 9% of Class B, components belonging to Classes C and D were not quantified (Angulo, Teixeira, Castro, & Nogueira, 2011).

In 2015, approximately 612 thousand tons of CDW were collected in the city of Belo Horizonte, representing a total of 42.7% of the quantity of waste destined in the city, generating a value between 0.24 and 0.26 tons of CDW per capita^{-1} year^{-1}. This research also presents the data related to the qualitative analysis carried out on the samples collected at the rubble recycling station. As a result, it can be seen that the gravimetric composition of the C&D waste produced in the city is composed mainly of Class A, such as blocks, mortars, and concrete, among others, representing 95.5% of the amount of waste generated, followed by class B waste, such as paper, plastic, glass, and metal, in a smaller proportion (Bessa, Mello, & Lourenço, 2019).

By evaluating the typology of the CDW dumped at the three recycling plants in Belo Horizonte, it was verified that a large part of the waste sent for recycling is based on ceramics and comes from residential rehabilitation works of houses (Carmo, Maia, & César, 2012).

An analysis of the percentages and origin of the CDW from the city of Passo Fundo/RS was carried out and excluded waste from land clearing; the highest collection rates were those from demolition and rehabilitation, which were 51.1% of the collected loads, as well as those from new constructions, that is, residential works and buildings under construction, which together represented 35.6%. Subtracting ground values from excavations, demolitions and rehabilitation represented 58.6%, and the new constructions were 41.4% (Bernardes, Thomé, Prietto, & Abreu, 2008).

In the city of Criciúma/SC, from 2001 to 2010, an estimated daily generation of CDW values between 0.62 and 1.46 kg per capita^{-1} day^{-1} was recorded. The average per capita generation of 0.96 kg day^{-1} emphasizes this value is within the range considered for Brazilian cities. In this same interval, 1,493,212.80 m² of the built area was recorded, representing an average annual waste generation of 22,398.19 tons in this period, corresponding to a daily generation of 93.33 tons, considering only 20 working days in each month. Equally important, the CDW generation values were obtained through an indicator second Pinto (1999), where production of 0.15 ton/m² of the built area is expected. The authors also emphasize that the recycling of CDW is of great importance to regional development since it contributes to the production of recycled aggregate, the conservation of natural reserves of sand and gravel, and the reduction of irregular discard areas. (Cardoso, Galatto, & Guadagnin, 2014).

The impasse over the reuse of CDW is mainly due to the scale of production required for processing the material in crushing plants to be viable and the prohibition of its disposal in sanitary landfills, per the legislation. In a big country like Brazil, with regional differences, it is difficult, if not impossible, for the same legislation to be applied in all municipalities. In practice, many sanitary landfills in the country receive this waste for use in the construction and maintenance of internal roads and, eventually, to cover the waste (Eigenheer & Ferreira, 2011).

A study on the application of recycled aggregates from CDW with cement and hydrated lime developed in Brazil, through the field and laboratory tests, proved that aggregates from CDW can be used as a sub-base material for pavements in places of heavy traffic; however, limited to municipal traffic, where most vehicles consist of single axels, dual/single axes, and dual tandem (Beja, Motta, & Bernucci, 2020).

CDW recycling plants may be economically viable for public authorities depending on market conditions, varying according to the circumstances of each municipality, such as final disposal costs in a sanitary landfill, expenses involved in the transport of waste to the landfill, as well as in the purchase price of natural products. However, viability also depends on the continuous operation and production capacity of recycling centers (Nunes, Mahler, Valle, & Neves, 2007).

The dimensioning of the space needed to install a CDW recycling plant (Jadovski, 2005) conducted technical visits to numerous plants in operation in Brazil, when it was found that the operation of such activity demands areas as stated by the values presented in Table 1.

Table 1- Area required for recycling plant depending on production capacity

Production capacity ton/h	Production capacity ton/year	Required Area m²
10	21,000	5,000
20	42,000	6,500
30	63,000	8,000
40	84,000	10,000
50	105,000	12,000
75	158,000	16,000
100	210,000	20,000

Within the scope of the city of Rio de Janeiro, electronic bidding was published to contract technical closure services with the maintenance of the CTR - Gericinó and implementation of a new cell to receive CDW collected by companies accredited by COMLURB, with a minimum capacity of 730,000 ton, over 5 years. Among the justifications, the prohibition of dumped CDW in the transfer stations is recognized, as well as the economic unfeasibility of carrying out the destination of them in the CTR - Rio in Seropédica, due to the great distance from this place to the generation center. It also recognizes that such conditions caused the dispersion of a large amount of rubble in inappropriate places, causing environmental impacts and burdening public coffers. Therefore, the most viable solution was the implementation and operation of a cell to dispose of this waste at the CTR – Gericinó because of the proximity to the west zone, as well as the availability of the area (Rio de Janeiro (Cidade), 2019).

Despite the attempt to contract these services, the Tribunal de Contas do Município do Rio de Janeiro - TCMRJ (Court of Auditors of the Municipality of Rio de Janeiro) suspended the bidding for the electronic bidding n° 382/2019. The rapporteur accepted the complaint against COMLURB. Among the allegations, it was requested the legal basis for the bidding to take place electronically, further clarifications on the closure of the Gericinó landfill due to it has not been in operation since 2014, as well as the legal basis for the provision of contracting a company with experience only in earthworks for the execution of sanitary work, supposedly complex (TCMRJ, 2019).

2. CDW QUANTIFICATION, GENERATION DATA PUBLISHED BY AUTHORITIES, APPLICATION OF STATISTICAL TESTS, AND MAPPING OF CDW DISPOSAL SITES

Initially, research was carried out with government agencies to provide information on the topic of interest, such as the Secretaria Municipal de Desenvolvimento Econômico, Inovação e Simplificação - SMDEIS (Municipal Secretary for Economic Development, Innovation, and Simplification), Secretaria Municipal de Planejaento Urbano - SMPU (Municipal Secretary for Urban Planning), COMLURB and SNIS.

2.1. Quantification of Construction Waste

Data collection was conducted to estimate the total construction waste produced in Rio de Janeiro, carried out through a survey on the page of the SMPU, and obtained through the tables referring to the issued licenses from 2006 to 2020. This information has been organized according to neighborhoods and city planning areas.

The estimate of CDW generation can be verified through consolidated generation indicators from three information bases, namely: the activities of the built area - services performed and effective losses, the movement of loads by transport companies, and the monitoring of discharges in the places used as the destination of the CDW. This method makes it possible to compose a quantification indicator by aggregating two important parts of urban construction activity: the formal construction of new buildings and the informal execution of rehabilitation and expansions.

The quantification of construction waste generated in the city was carried out by adopting the generation indicator second Pinto (1999), whose estimate is obtained from licensed construction activities, which indicate values of waste products in a formal building through the use of construction waste generation rate in the order of 150 kg/m² built (Pinto, 1999).

2.2. Generation Data Published by Authorities

Information from COMLURB was used about the quantity of CDW produced in the municipality of Rio de Janeiro since this company is responsible for the inspection and management of urban solid waste in the city. Another source of information used was the diagnosis of urban solid waste management written by SNIS.

2.3. Application Statistical Tests

The results from the quantification of the generation of construction waste second to Pinto's methodology (1999), as well as the survey of the total production of waste published by the agencies COMLURB and SNIS, will be analyzed initially through the ANOVA statistical test, considering two periods, the first from 2006 to 2020 and the second from 2011 to 2020. Furthermore, for statistical analysis between two data groups, the T-test will be used, as this methodology is applied when two data samples are collected, whose sample mean values calculated may differ (Field, Miles, & Field, 2012).

The choice of the generation data, which will be used to calculate the proposed area for the implementation of the construction waste recycling plant, it will be the data group that obtains the best representation following the results from statistical tests, which will be calculated through the software Minitab version 16.

2.4. Mapping Locations C&D Waste Destination

Information was obtained from SMDEIS of the city of Rio de Janeiro about the places licensed to carry out waste disposal in the municipality.

The representation of the maps, as well as the proposition of adequacy of the reception locations and creation of the recycling plants of the CDW, will be illustrated through mapping using the QGIS software version 3.16.19. For this representation, the CBERS 04A satellite images will be geoprocessed, whose raster files have a spatial resolution of 2.00 m for the panchromatic spectral band and 8.00 m for the multispectral band. In this process, images of colored composition will be produced through the spectral bands red, green, and blue. Later, the procedure will be pansharpening between the color composition and the raster file of the panchromatic spectral band to obtain an image of spatial resolution of 2.00 m.

The area calculation for choosing the location to propose the installation of a CDW recycling plant will be carried out according to the criteria adopted by (Jadovski, 2005). As a parameter for determining the space, the data presented in Table 1, therefore the verification of those values will be carried out to obtain the Pearson linear correlation coefficient (r), as well as to discover the equation of the fitted line. In addition to this requirement, the availability of vehicle access roads will be verified, and the delimitation outside the permanent preservation areas following the legislation (Brasil, 2012).

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Waste Generation Second City Planning Area

The quantification of construction waste was done by a survey of the total built area obtained through the licenses concession carried out, according to the information on the SMPU from the city of Rio de Janeiro, between the period from 2006 to 2020. Figure 1 illustrates the total area of occupancy concession as stated by the planning area of the city.

3.1.2. Generation of CDW Released By Authorities

A survey was carried out about the generation of CDW produced in Rio de Janeiro, published by control authorities from 2006 to 2020. Table 2 presents the data released by COMLURB, while table 3 summarizes information from SNIS through publications on the diagnosis of urban solid waste management.

3.2. Discussions

3..2.1 ANOVA Statistical Test Period 2006 to 2020

The ANOVA statistical test was applied for the period between 2006 and 2020, establishing a confidence interval of 95%; the results of this analysis are shown in table 6.

Table 6 - Statistical indicators of construction waste generation 2006 to 2020

Source	Average (ton)	Standard deviation (ton)
COMLURB	303,537.00	228,681.00
Estimate	361,992.00	156,001.00
SNIS	381,440.00	247,439.00

Source: Authors (2022).

The mean values of the three groups of data are close, and this interpretation can be validated as maintained by the result of the ANOVA test, where a P value equal to 0.589 was obtained, therefore, greater than the significance index, which is 0 .05, thus, hypothesis H_0 should not be rejected. Therefore, it can be attested with a significance level of 5% that the average values of CDW generation for the three data groups are the same.

3.2.2 ANOVA Statistical Test Period 2011 to 2020

The ANOVA statistical test was applied for the period from 2011 to 2020, establishing a confidence interval of 95%, the results of this analysis are shown in table 7.

Table 7 - Statistical indicators of construction waste generation 2011 to 2020

Source	Average (ton)	Standard deviation (ton)
COMLURB	170,980.00	130,071.00
Estimate	382,527.00	184,419.00
SNIS	246,119.00	166,158.00

Source: Authors (2022).

According to the ANOVA statistical test result, the P-value equal to 0.022 was obtained, therefore, lower than the significance index, which is 0.05. Thus, hypothesis H_0 must be rejected, and hypothesis H_1 must be accepted. Hence, it can be assured with a significance level of 5% that the average values of CDW generation for the three data groups are not the same.

The statistical calculation of the Tukey method was applied, considering the confidence interval of 95%, to determine the probable intervals of the differences and evaluate the practical significance between these discrepancies. The results of the analysis are presented in Figure 2, therefore, according to what can be observed in the lines in the red shade, the significant difference is between the averages from COMLURB and Estimate since the confidence interval for the estimate does not exceed the red line. As a result, there is no significant difference between the averages from COMLURB and SNIS and between the averages from Estimate and SNIS.

3.2.3 Application Test T Generation CDW Estimates and Movement of Collecting Companies 2006 to 2020

As it was verified that there is no significant difference between the average values of the construction waste generation estimate and the data from SNIS, the T-test was applied to the data group related to the estimate and the quantities destined by the collecting companies and self-employed hired by the generator, which was added to the total waste destined by the generator itself, second information presented in columns 3 and 4 of table 3.

As a P value greater than 0.1 was obtained, therefore, higher than the significance index, which is 0.05; hence, the H_0 hypothesis should not be rejected. Thus, it can be affirmed with a significance index of 5% that the construction waste generation values from 2006 to 2020, referring to the estimation method, and the data released by the SNIS follow a normal curve. Furthermore, variances related to the two groups of data were also checked. The result verified for the P F test value was 0.228. Therefore, higher than the significance index, which is 0.05, it can be attested with a significance index of 5% that the variances for the two data groups can be considered the same.

As the conditions regarding the obligation for the data to follow a normal distribution were met, as well as the variances to be considered the same, the T-Test was applied, whose result indicated a P value equal to 0.399, thus, higher than the significance index of 0, 05, therefore, should not be rejected H_0, so it can be confirmed according to the 5% significance index that the average values of CDW generation for the two data groups are the same.

3.2.4 Application Test T Generation CDW Estimates and Movement of Collecting Companies 2011 to 2020

As it was verified that there is no significant difference between the estimated average quantities for construction waste generation and the average of the data from SNIS, the T-test was applied to the group of estimated and the quantities destined by collecting companies and self-employed hired by the generator, which was added to the total waste destined by the generator itself, second information presented in columns 3 and 4 of table 3, in other words, the amounts of rubble that were destined under the responsibility of the city hall were subtracted.

According to the normality test results, the P value was higher than 0.1, thus, greater than the significance index, which is 0.05, so hypothesis H_0 should not be rejected. Thus, it can be attested with a significance index of 5% that the construction waste generation values for the period from 2011 to 2020, referring to the estimation method, as well as the data from SNIS describe a normal curve.

Verifying the variances related to the two data groups was carried out, establishing a 95% index as a confidence interval. The result verified for the P value from the F test was 0.450, therefore, higher than the significance index, which is 0.05, thereby hypothesis H_0 should not be rejected, hence can be attested with a significance index of 5% that the variances for the two groups of data can be considered the same.

Finally, the T-Test was calculated, the result of which showed a P-value equal to 0.014, thus, lower than the significance index of 0.05, therefore, H_0 must be rejected, and the alternative hypothesis H_1 must be accepted, therefore, can be confirmed according to the 5% significance index that the average values of construction waste generation for the two data groups are statistically different.

After applying statistical tests and finding the data that represented the best indicators, it was decided to select the reference for generating construction waste for the period from 2006 to 2020, obtained by estimating second Pinto's methodology (1999), whose average amount of production was 361,992.00 ton year^{-1} and standard deviation of 156,001.00 ton year^{-1}, as shown in Table 6.

3.2.5. Mapping for C&D Discard

As stated by Rio de Janeiro (Cidade) (2021), for the adequacy of the final destination of solid waste in the city, a concession contract was signed between COMLURB and the company Ciclus Ambiental do Brasil S.A until 2026, which can be extended for five years, the contractual scope includes the implementation, operation and maintenance of the waste transfer stations, and the landfill in Seropédica. Figure 3 illustrates the logistics for transferring the waste from the ETRs to the CTR – Rio in Seropédica. As can be seen, there are five waste transfer stations in the city, the ETR – Caju in AP1, the ETR – Marechal Hermes in the AP3, the ETR – Jacarepaguá in the AP4, and the ETRs Bangu and Santa Cruz in the AP5.

It should be noted that SMDEIS licenses the transfer stations. However, according to the information presented in table 4, the activity regulated by the environmental agency is for the operation of a solid urban waste transfer station. Hence, it is not described in the activity of ETRs the receipt of CDW. Although the responsible environmental agency regulates the ETRsmoreover the process related to the Jacarepaguá unit was started on February 18, 2011, according to a consultation on the progress of processes at the in Secretaria Municipal de Meio Ambiente (Municipal Environment Department) (SMAC, 2011). At the time, the extension of the operating license for the activity of a recycling and composting plant was required, according to a requirement published on February 12, 2019, issued by the municipal environmental agency, when it was required to submit an application for the operation of a waste transfer station since the activity licensed at the time was not the same (SMAC, 2019).

As for the Seropédica sanitary landfill, this company is regulated by the Instituto Estadual do Ambiente – INEA (State Institute of the Environment), whose information is presented in table 5. According to what can be seen, the activities of the sanitary landfill regulated by the environmental agency include the disposal of Class II waste of residential, commercial, and industrial origin. In this way, the activity of receiving CDW is not considered the object of the license. Although such activity is not described in the object of the sanitary landfill operating license, the charge for disposal of inert solid waste is allowed at CTR-Rio, in Seropédica, as written in Item 4A of the table of values of the special services to be charged and collected by COMLURB, established in § 1º of Art. 1° from the regulation (Rio de Janeiro (Cidade), 2021).

In this way, it can be affirmed that the sanitary landfill receives inert solid waste, which includes construction waste; in this way, in disagreement with the object of the operating license issued by the environmental agency, it must be emphasized that in the "§ 1º of Art. 4º from CONAMA 448 establishes that construction waste cannot be sent to urban solid waste sanitary landfills (Brasil, 2012).

Reaffirming the point of view defended by (Eigenheer & Ferreira, 2011), where they describe that in practice, many sanitary landfills in the country receive construction waste for use in the construction and maintenance of internal roads and, eventually, in the coverage. Because of the above justifications, adopting a new management model for the CDW produced in Rio de Janeiro is necessary. Therefore it is suggested that the ETRs managed by COMLURB be adapted to enable the receipt of this class of waste temporarily for later sending of the CDW to the recycling plant that will be created.

The process of adapting the transfer station should start primarily in the ETR of Jacarepaguá since Planning Area 4 was the region where 50.70% of the total estimated construction waste in the city was generated from 2006 to 2020, as shown in figure 1. In the second order of priority, the ETR of Marechal Hermes must be adequate Planning Area 3 was the region that produced 17.66% of the calculated waste in the municipality of Rio de Janeiro. In the third order of priority, the adaptation of the ETR of Bangu is suggested because, in Planning Area 5, 16.59% of the total rubble originated in the city. Finally, it is recommended to adapt the ETR of Caju, a strategic plan to receive waste from Planning Area 2 and Planning Area 1, regions where it was estimated the generation of 8.28% and 6.77%, respectively, of the number of materials produced in the city of Rio de Janeiro.

In addition to adapting the transfer stations, it will be necessary to build a CDW recycling plant so that this unit to be the destination of the waste sent to ETRs; in this way, it will be possible to recycle the rubble. For the sizing of the CDW recycling, an estimation method was used considering the generation of waste for the period from 2006 to 2020, whose average production value was 361,992.00 tons year^{-1} and a standard deviation of 156,001.00 tons year^{-1}, according to data presented in Table 6.

The choice of this reference value was possible after applying the ANOVA statistical test between the generation data from COMLURB, estimated according to the methodology of Pinto (1999), and generation values published by SNIS. As a result, obtained in Section 3..2.1, the construction waste generation data were statistically equal, according to the 95% confidence interval. However, when this test was carried out for the period between 2011 and 2020, as maintained by the same confidence interval, it was proved that the mean values of these three data groups are statistically different, per the results presented in section 3.2.2. After verifying the existence of statistical difference, Tukey's statistical method was applied when it was identified that a significant difference occurred between the generation values published by COMLURB and the estimation metric second Pinto (1999). However, there was no significant difference between the estimate and data from SNIS, according to the results illustrated in Figure 2. For this reason, as there was no significant difference between the average values estimated second Pinto's method (1999) and data published by the SNIS, these two data groups were selected for a more detailed statistical analysis. Therefore, the CDW generation values from COMLURB were eliminated.

As two data groups were used for statistical analysis, the T-test was applied considering two periods, from 2006 to 2020; no difference was identified, in accordance with the results presented in Section 3.2.3. However, for the interval between 2011 and 2020, the existence of a significant difference was proven, according to data shown in Section 3.2.4.

The hypothesis found to justify the existence of a statistically significant difference between the analyzed data is due to the change in the waste management system in the city of Rio de Janeiro; as presented in the introductory section, the CTR – Rio in Seropédica was inaugurated on 20 April 2011 (Rio De Janeiro (Cidade), 2021).

It should be noted that the current urban solid waste management model was gradually regularized. According to information presented in table 4, the beginning of the validity of the municipal operating licenses of the ETRs has a date posterior to the inauguration of the CTR - Seropédica, being on April 16, 2012, to ETR Marechal Hermes, on June 20, 2012, to ETR Santa Cruz, on April 9, 2014, to ETR Caju, on May 9, 2014, to ETR Bangu and on June 17, 2021, to ETR Jacarepaguá.

Regarding the ETR of Jacarepaguá, as presented in the introductory section, this unit was where the waste collected in the city had been dumped since the beginning of the sanitary landfill operation (Rio de Janeiro (Cidade), 2011). However, according to Table 4, the concession of the operating license for the solid urban waste transfer station activity was only completed on July 17, 2021. Because of this, it can be attested that the ETR of Jacarepaguá operated in disagreement with the activity licensed since the beginning of its operation.

As a consequence of the beginning of operation of the CTR – Rio in Seropédica, farther from the urban center of the city of Rio de Janeiro, to the metropolitan landfill of Jardim Gramacho, as well as the CTR Gericinó, disabled in June 2012 and April 2014, respectively. In addition to the legal impossibility of dumping the CDW in the ETRs, this activity is not being described in the objects of the operating licenses resulted in the dispersion of this waste class. This fact was verified through the statistical tests and discussed in this chapter. Therefore, adopting the proposed management model is recommended for the reasons explained.

Because of the above, the criterion for determining the area was used to carry out the sizing of the CDW recycling plant that meets the generation demand of 361,992.00 tons year-1, according to data presented in table 6, under the data disclosed in table 1. However, as the waste production estimate exceeds 210,000.00 tons year^{-1}, these values were verified to discover the Pearson (r) linear correlation coefficient and obtain the adjusted straight-line equation, as illustrated in Figure 4.

According to what can be verified, the value of R^2 was 0.9987, thus representing a probability of 99.87%. As for the value of Pearson's linear correlation coefficient obtained, it was 0.9993. Thus it is concluded that there is a strong relationship since this result is very close to 1 (one). Because of the above justifications, it can be attested that the equation of the adjusted line is a good representation of data, as a result of applying the equation, a total area of 32,397.50 m² was measured, so it is proposed to adopt an area with this dimension, also selected according to criteria for the availability of access roads for trucks, as well as the delimitation outside the permanent preservation areas, established following the legislation (BRASIL, 2012), as illustrated in the Figures 5 e 6.

Finally, regarding the proposed model for the management of construction waste in Rio de Janeiro, in line with the criteria presented, the adequacy of the ETRs managed by COMLURB is suggested to enable the reception of waste temporarily. Afterward, sending to the recycling plant, the proposition of this mold is illustrated in Figure 7.

4. CONCLUSIONS

Achieving the purpose of this study, which consists of proposing a new management model for construction waste in Rio de Janeiro, initially, estimates of construction waste were carried out, second to the city planning area.With regard to statistical tests, it was confirmed by the ANOVA test that there was no statistically significant difference between the generation data published by COMLURB, estimated according to Pinto's methodology, and values published by the SNIS for the period from 2006 to 2020, as maintained by the confidence interval the 95% index. However, when this test was performed for the period from 2011 to 2020, it was verified that the mean values of the three data groups are statistically different,. It was also confirmed, through the Tukey method, that a significant difference occurred between the generation data published by COMLURB and the estimation metric developed by Pinto (1999), .

The values estimated according to Pinto's metric (1999) and data published by the SNIS, were analyzed through the T-test for the period from 2006 to 2020, and no difference was identified. However, from the time between 2011 and 2020, the occurrence of a significant difference was proven.

As for the mapping of authorized sites for the disposal of waste produced in the city of Rio de Janeiro, it was attested that the transfer stations managed by COMLURB, cannot receipt of CDW . It was also found that the ETR of Jacarepaguá operated in disagreement with the activity licensed since the beginning of its operation, due to the granting of the license to operate as a solid urban waste transfer station was concluded only on July 17, 2021.

Regarding the Seropédica sanitary landfill, the charge to dispose of construction waste in the CTR – Rio in Seropédica is allowed, as written in item 4A of the table of values of the special services to be charged and collected by COMLURB, established by § 1º of Art. 1° from regulation N°3-R (Rio De Janeiro (Cidade), 2021). As a result, it can be said that the sanitary landfill receives inert solid wastes, , in disagreement with the object of the operating license issued by the environmental agency, as well as the "§ 1º of Art. 4º from CONAMA 448stablishes that CDW cannot be sent to urban solid waste sanitary landfills (BRASIL, 2012).

For the implementation of the CDW recycling plant, it was found that adaptation of ETRs must start primarily at the ETR of Jacarepaguá since the AP4 corresponded to 50.70% of the total estimated. In the second order of priority, it was recommended to adapt the ETR of Marechal Hermes, how AP3 represented 17.66% of the amount of waste calculated. In the third order of priority, the adjustment of the ETR of Bangu was suggested because the AP5 corresponded to 16.59% of the estimated materials volume. Finally, an adaptation of the ETR of Caju was suggested, as it is a strategic place to receive waste from AP2 and AP1, regions where the generation of 8.28% and 6.77%, respectively, of the total CDW estimated in the city of Rio de Janeiro.

In addition to that, the need to create a construction waste recycling plant was presented, to this end, it is necessary 32,397.50 m² of area available, access roads for trucks, as well as delimitation outside the permanent preservation areas. In conclusion, a new model was proposed for the management of construction waste in the city of Rio de Janeiro.

Finally, it is recommended to revoke the § 1° of Art. 17 from regulation “N” COMLURB N°002 of February 3, 2022, since it is proven that the site has an operating license compatible with the activity, there is no technical justification for prohibiting the disposal of waste from large generators, in companies regularized by other inspection and control institutions.

5. DECLARATIONS

5.1. Study Limitations

No limitations were known at the time of the study.

5.2. Acknowledgements

Not applicable.

5.3. Funding source

This research was funded by the authors.

5.4. Competing Interests

No conflict of interest exists in this publication.

5.5. Open Access

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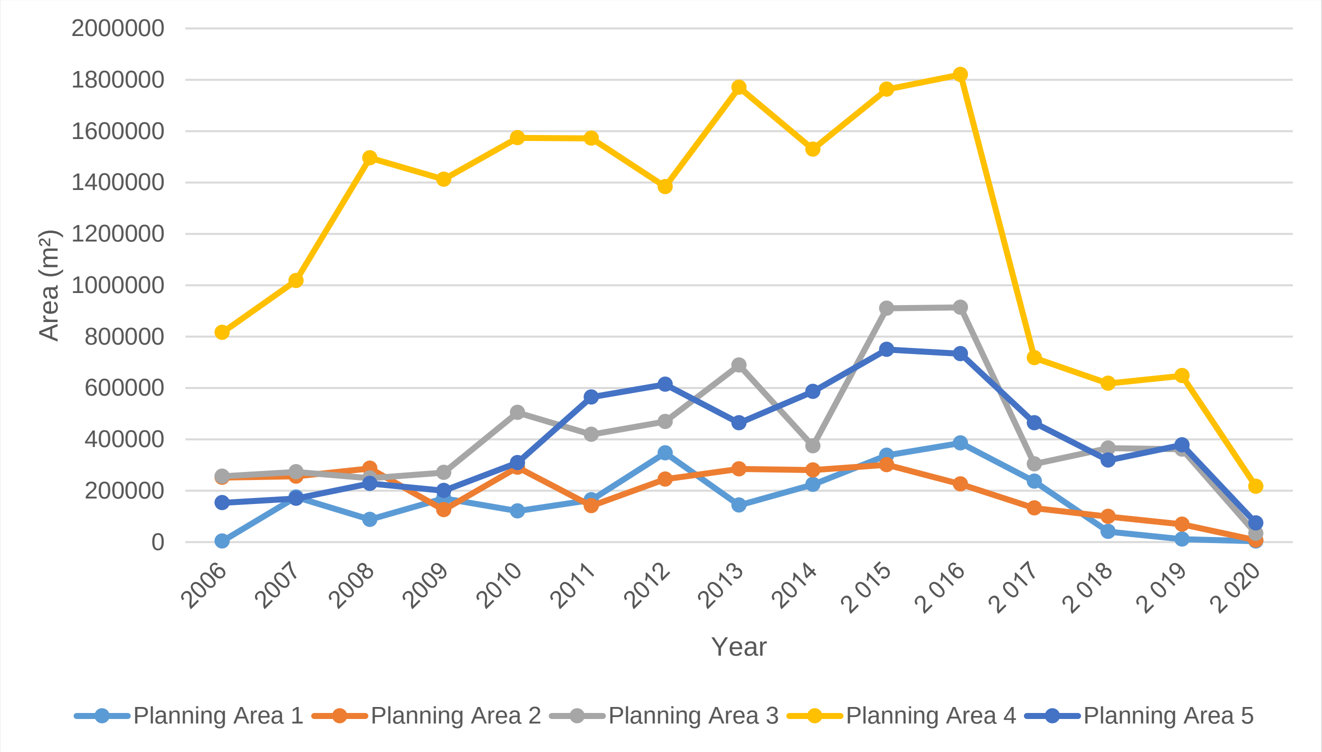


Figure 1 - Total area of occupancy concession according to the Planning Area

Source: Authors based on SMPU data (2006 - 2020).

Table 2 - Amount CDW generated in the city of Rio de Janeiro according to COMLURB

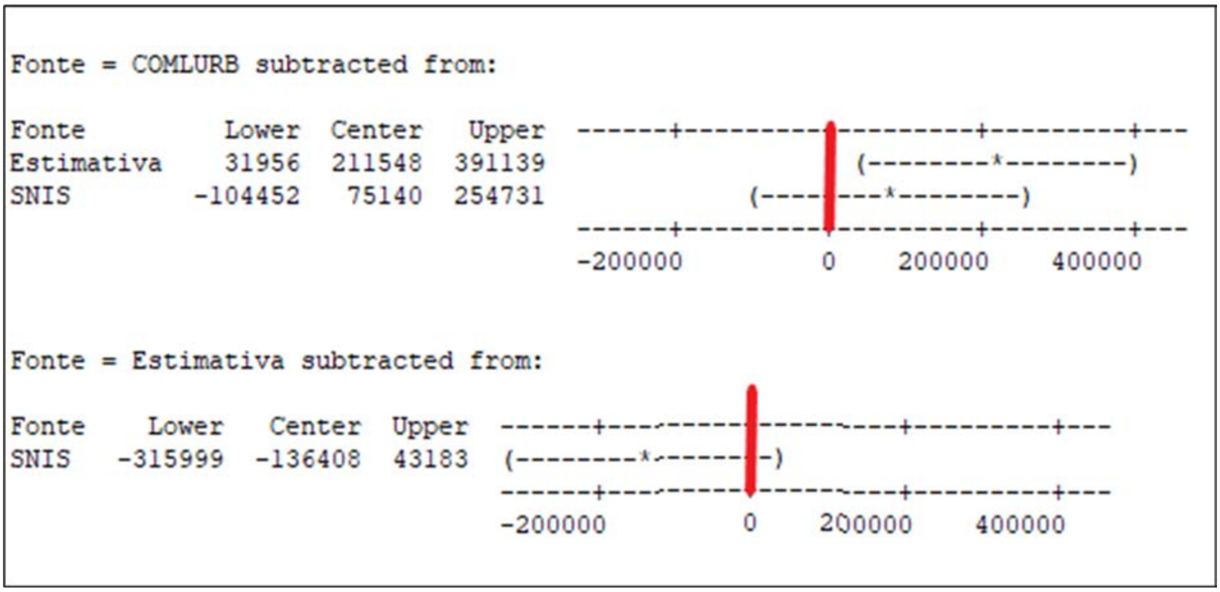
Year	Total weight (ton)
2006	678,258.00
2007	514,475.00
2008	640,559.00
2009	615,647.00
2010	394,324.00
2011	419,225.00
2012	316,295.00
2013	212,687.00
2014	229,202.00
2015	217,079.00
2016	104,285.00
2017	76,883.00
2018	66,209.00
2019	19,036.00
2020	19,037.00

Source: COMLURB (2021).

Table 3 - *Amount CDW generated in the city of Rio de Janeiro according to SNIS*

Year	City Hall or collected by it (ton)	Companies and self-employed hired by the constructor (ton)	constructor (ton)	Total weight (ton)
2006	105,410.10	388,788.60	54,555.40	548,754.10
2007	78,501.00	377,350.00	58,623.00	514,474.00
2008	123,998.33	688,119.39	0.00	812,117.72
2009	112,221.90	559,840.60	0.00	672,062.50
2010	124,005.70	588,996.00	0.00	713,001.70
2011	102,576.00	489,391.00	0.00	591,967.00
2012	91,292.00	321,100.00	0.00	412,392.00
2013	86,392.00	212,687.00	0.00	299,079.00
2014	70,549.00	229,202.00	0.00	299,751.00
2015	68,634.00	217,079.00	0.00	285,713.00
2016	65,715.00	104,294.00	0.00	170,009.00
2017	57,849.00	74,709.00	0.00	132,558.00
2018	48,882.00	66,225.00	0.00	115,107.00
2019	49,238.00	48,896.00	28,840.00	101,018.00
2020	19,036.00	34,564.00	0.00	53,600.00

Source: SNIS (2006-2020).



```
Fonte = COMLURB subtracted from:

Fonte          Lower  Center   Upper  ------+---------+---------+---------+---
Estimativa     31956  211548  391139                     (--------*--------)
SNIS         -104452   75140  254731               (--------*--------)
                                      ------+---------+---------+---------+---
                                      -200000         0    200000    400000

Fonte = Estimativa subtracted from:

Fonte     Lower   Center  Upper  ------+---------+---------+---------+---
SNIS    -315999  -136408  43183  (--------*--------)
                                 ------+---------+---------+---------+---
                                 -200000         0    200000    400000
```

Figure 2 - Tukey test result data construction waste generation period from 2011 to 2020

Fonte: Autors (2022).

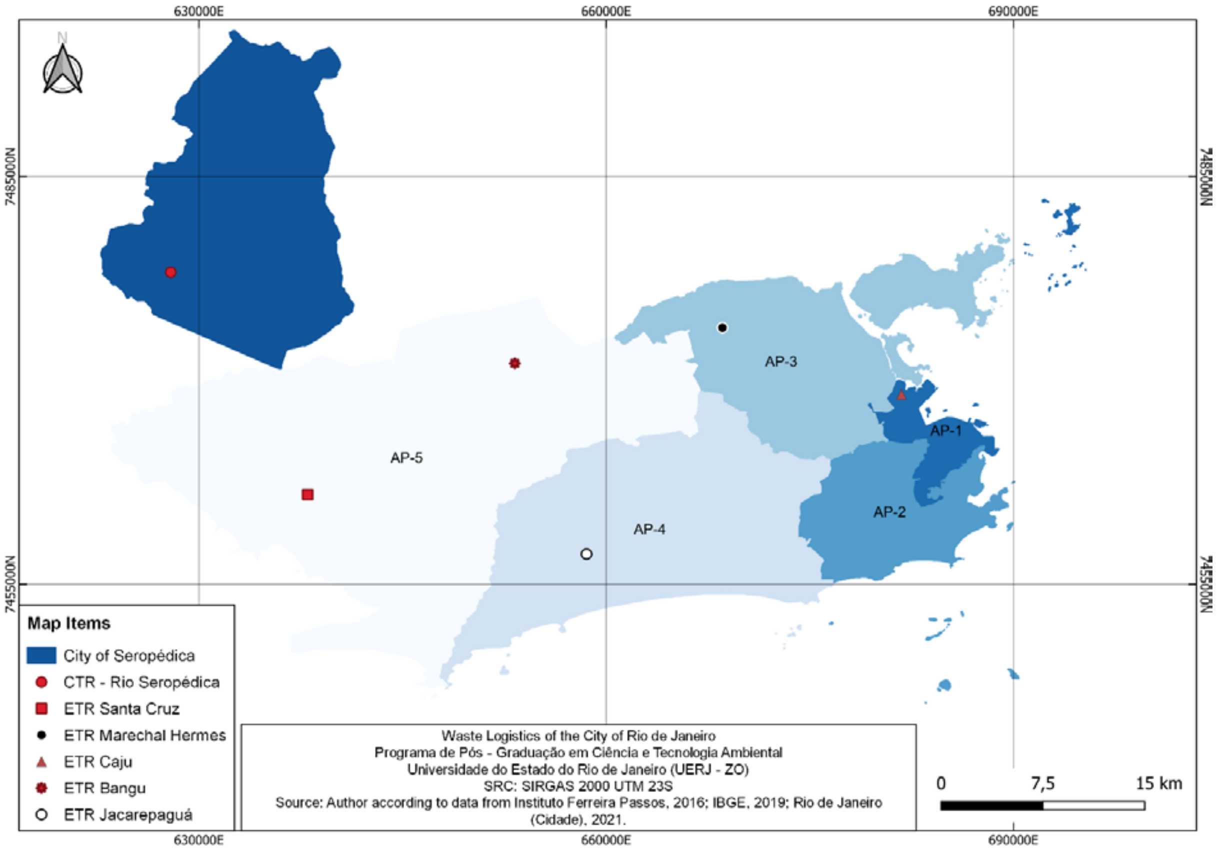


Figure 3 - Waste transfer logistics to CTR - Rio in Seropédica

Source: Authors according to information (Rio de Janeiro (Cidade), 2021).

Table 4 - Municipal Operating Licenses for COMLURB ETR

Process number	**Requester**	**Summary description**	**document number**	**Date of document**
142011952010	ETR - Marechal Hermes	Solid Urban Waste Transfer Station	LMO 000692	04/16/2012
142011962010	ETR - Santa Cruz	Solid Urban Waste Transfer Station	LMO 000732	06/20/2012
		Solid Urban Waste Transfer Station with an operational capacity of 21,000 ton/month	LMO 2862	10/20/2021
142007702011	ETR – Caju	Solid Urban Waste Transfer Station with operational capacity for 4,000 ton/day	LMO 001229	04/09/2014
142000192014	ETR - Bangu	Solid Urban Waste Transfer Station with an operating capacity of 3,000 ton/day	LMO 001244	05/09/2014
142004942011	ETR - Jacarepaguá	Solid Urban Waste Transfer Station with an operational capacity of 20,000 ton/month	LMO 2793	06/17/2021

Source: Authors according to data (SMDEIS, 2022).

Table 5 - Operating License CTR - Seropédica

Process number	**Requester**	**Summary description**	**document number**	**Date of document**
EXT-PD/ 014.10456/2021	Ciclus Ambiental do Brasil S.A. (CTR - Seropédica)	Sanitary landfill (up to 10,400 t/d) in an area of 989,215.11 m², belonging to sub-landfill 1 and sub-landfill 3 (stages 1A, 1B1, 2A1, 2A2 and 3A), for disposal of class II waste of residential, commercial and industrial origin; Slurry Treatment Station (primary, secondary, tertiary) and Treatment of slurry by reverse osmosis; treated effluent discharge line; workshop and point of departure supply, biogas capture and burning system (04 centrifugal blowers and 05 burners)	LO Nº IN011445	02/08/2022

Source: Authors according to data (INEA, 2022).

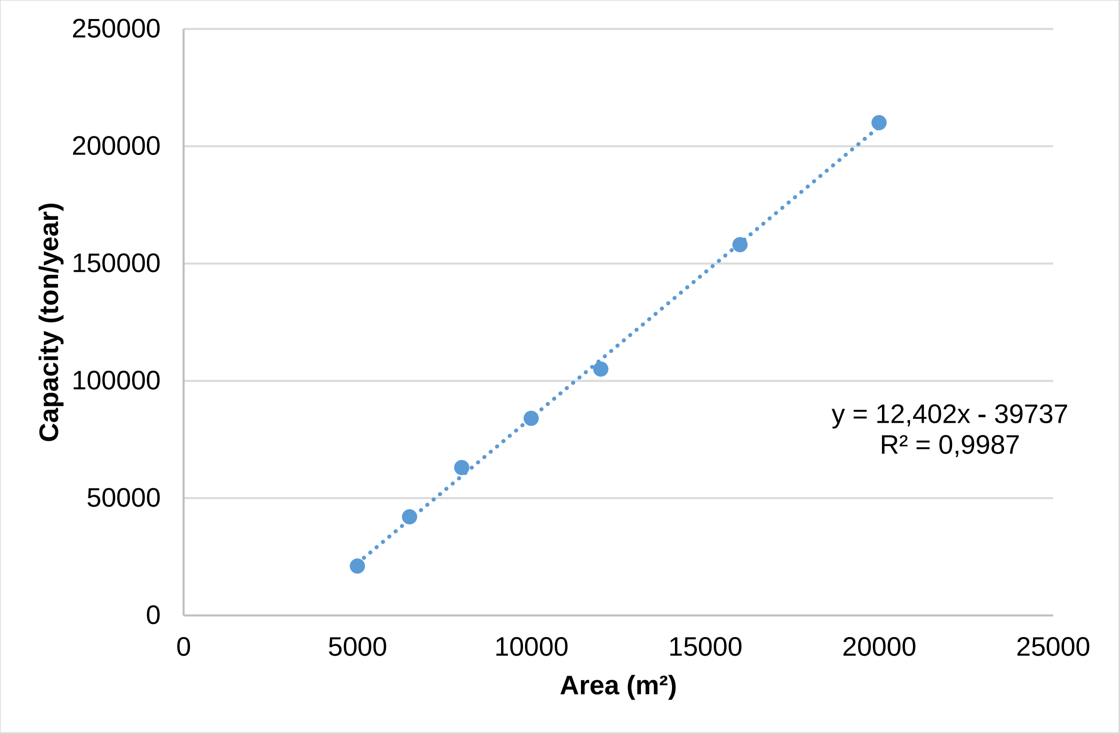


Figure 4- Area calculation parameter for CDW recycling plant

Source: Authors according to data (JadovskI, 2005).

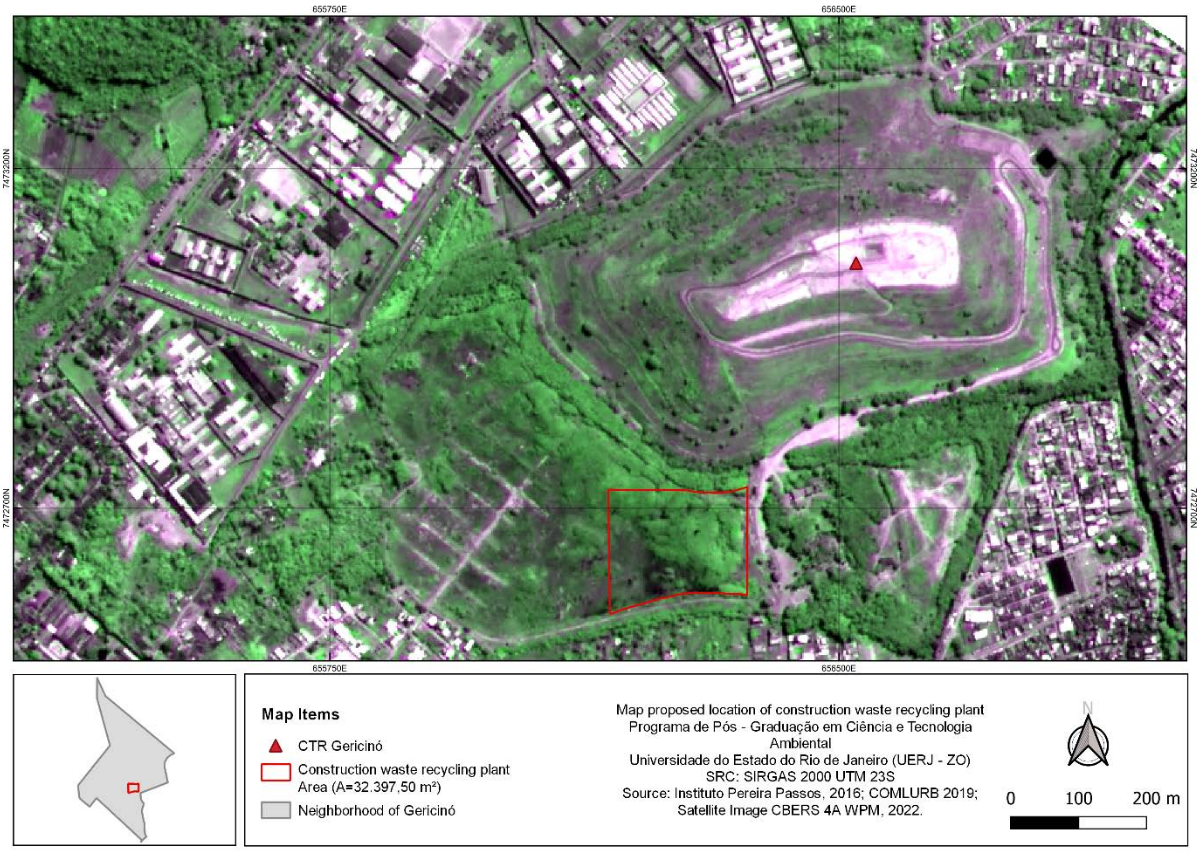


Figure 5- Proposed area for the creation of construction waste recycling plant

Source: Authors (2022).

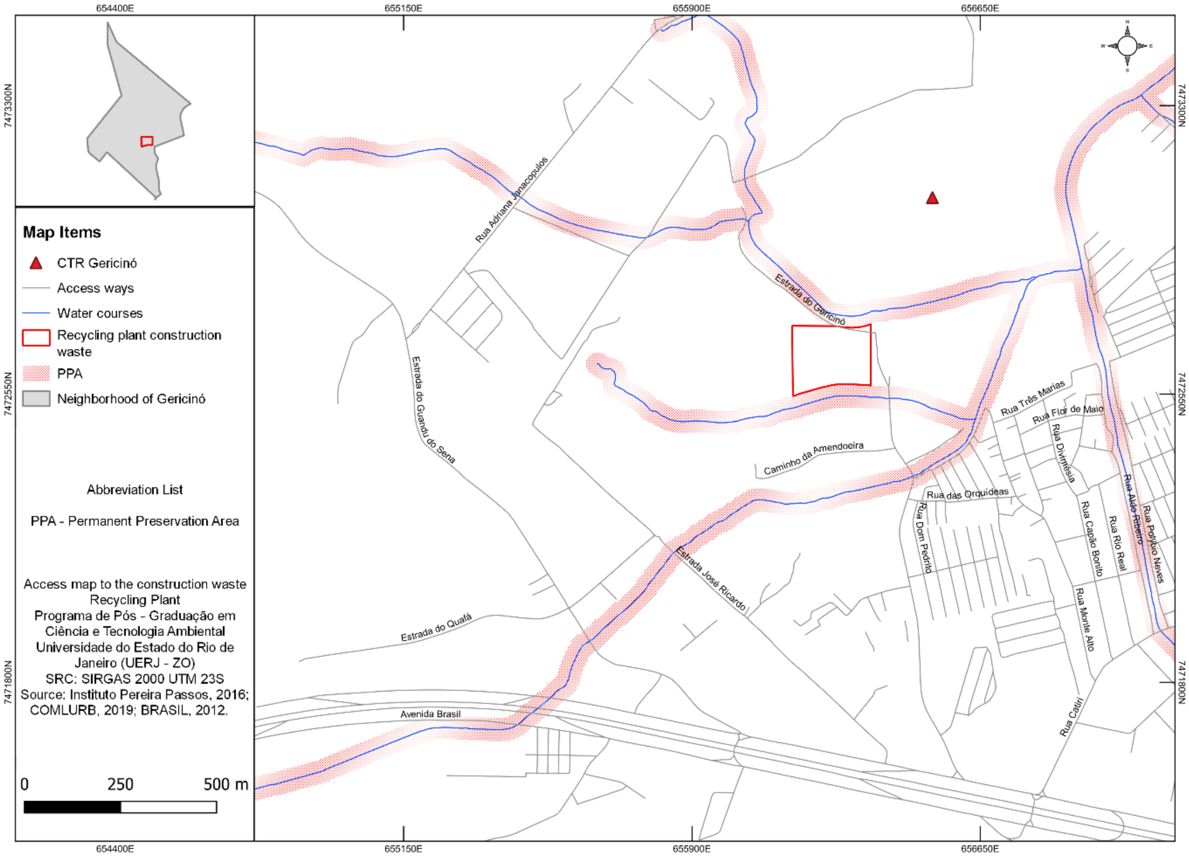


Figure 6 - Access to the construction waste recycling plant according to selection criteria

Source: Authors (2022).

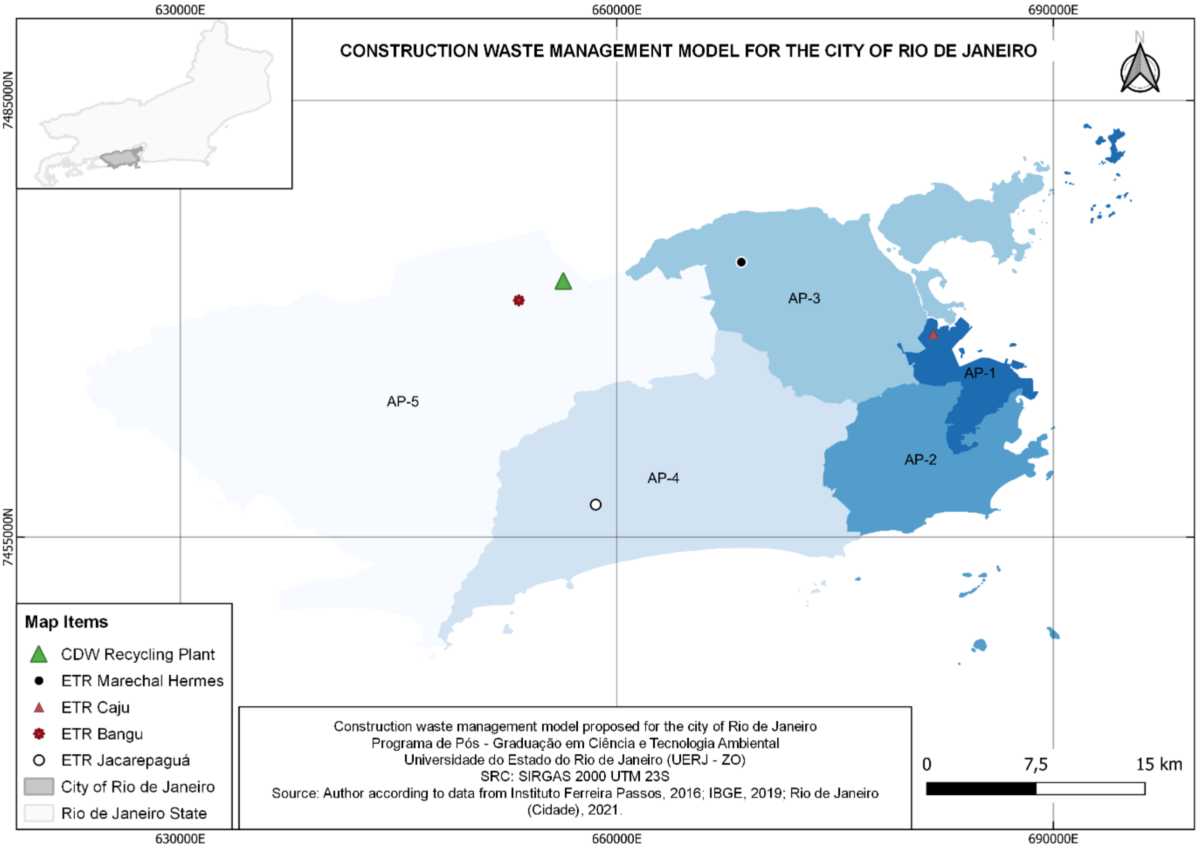


Figure 7- Construction waste management model for the city of Rio de Janeiro

Source: Authors (2022).

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ERRATUM

OPTIMIZATION AND KINETICS STUDIES OF THE DISSOLUTION OF DOLOMITE IN SULPHURIC ACID (H_2SO_4) VIA BOX-BEHNKEN EXPERIMENTAL DESIGN

JOSEPH, Isaac Adekunle[1*]; AJALA, Elijah Olawale[1]; AHMED, El-lmam Amina[2]; AJALA, Mary Adejoke[1]

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ERRATUM

At the SOUTHERN JOURNAL OF SCIENCES vol.30, n°33. 2022.

Page: 69

Where it reads: *Dolomite is a naturally occurring double carbonate mineral of magnesium and calcium with the chemical formula* $MgCa(CO_3)_2$ *(Baba et al., 2014; Pultar et al., 2018; Ajala et al., 2019). Dolomite ore can be used directly as an accelerating agent for cement hydration in concrete and a neutralizing agent in acidic soil (Mubaroka and Kurniawanb, 2015; Ajala et al., 2019). In addition, dolomite is rich in magnesium. Hence it can be used for the production of magnesium-based compounds such as magnesite* ($MgCO_3$)*, magnesium oxide (MgO), magnesium sulfate, as well as calcium sulfate, commonly known as gypsum* ($CaSO_4$)*. The abundant dolomite of Nigeria is spread across Abuja and the following states: Kogi, Oyo, Yobe, Kwara, Edo, and Nasarawa (Mookah and Abolarin, 2005)*.

Read: *Dolomite is a naturally occurring double carbonate mineral of magnesium and calcium with the chemical formula* $MgCa\ (CO_3)_2$ *(Baba et al., 2014; Pultar et al., 2019; Ajala et al., 2019). Dolomite ore can be used directly as an accelerating agent for cement hydration in concrete and a neutralizing agent in acidic soil (Mubaroka and Kurniawanb, 2015; Sivrikaya, 2018; Ajala et al., 2019). In addition, dolomite is rich in magnesium. Hence it can be used for the production of magnesium-based compounds such as magnesite* ($MgCO_3$)*, magnesium oxide (MgO), magnesium sulfate, as well as calcium sulfate, commonly known as gypsum* ($CaSO_4$)*, (Yildirim et al., 2010; Sivrikaya, 2018). The abundant dolomite of Nigeria is spread across Abuja and the following states: Kogi, Oyo, Yobe, Kwara, Edo, and Nasarawa (Mookah and Abolarin, 2005).*

The electronic copy of the manuscript was update and this modification was included in the file.

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SOUTHERN JOURNAL OF SCIENCES ANNUAL TRANSPARENCY REPORT

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Accepted 19 December 2022

In 2022 The SOUTHERN JOURNAL OF SCIENCES introduced the practice of producing an annual transparency report to give authors and institutions access to useful information about the journal. It was chosen to present the main facts in the format of bullets to make the report succinct.

- **Current Editor-in-Cheife**: Dr. Walter José Pelaez.
- **Past Editors**: Dr. Lavinel G. Ionescu; Dr. Luis A. B. De Boni.
- **Currently Edited by:** Dr. D. Scientific Consulting (CNPJ # 34.958.677/0001-59) - (Luis Alcides Brandini De Boni). A Brazilian Micro Company.
- **Number of countries represented in the journal council**: 12.
- **Number of conferences the journal was invited to publish the resulting material**: 3.
- **Number of conferences that the journal published the resulting material**: 1. (SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY - INTERNATIONAL VIRTUAL CONFERENCE. https://www.21scon.org)
- **Number of manuscripts received in 2022**: 24
- **Number of manuscripts published in 2022**: 11
- **Amount of manuscripts that will continue the publication process in 2023**: 4
- **Amount of improper submissions**: 6
- **Amount of rectified submissions**: 3
- **Innovative tools introduced in the journal**
    - *Abstract Maker tool*: <https://www.sjofsciences.com/Abstract-maker.php>
    - *Manuscript Sketch Tool*: <https://www.sjofsciences.com/manuscript-sketch-maker.php>
    - *Reference formatting tool**: <https://www.sjofsciences.com/doi_to_apa.htm>. This tool reflects the page https://citation.crosscite.org/
    - *Use of avatars (sample)*: https://youtu.be/iIMVj6FLmMg
- **Financial support received from other institutions (in USD):** $ 0,00. A proudly independent journal.
- **Indexed in:** Index Copernicus, Latindex, and I2OR.

DOI: 10.48141/SJS.v30.n34.2022.07_ATR_pgs_67_67.pdf .

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SOUTHERN JOURNAL OF SCIENCES – CALL FOR PAPERS

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Accepted 19 December 2022

As the world is going through a difficult period, the Southern Journal Sciences would like to make its part to improve it through scientific divulgation. Therefore, for **2023** all the manuscripts dealing with **food, drug, or energy production** will be published without publication fees as long as they follow the journal instructions on the website.

Including, **but not limited to**:

- raw-materials;
- production;
- quality-control;
- shelf life;
- energy efficiency;
- Production of fertilizers;
- biotechnology for food and drugs;
- industrial process.

We look forward to hearing from you and are grateful for your support in sharing this message with your friends.

Thank you very much.

Editors.

DOI: 10.48141/SJS.v30.n34.2022.07_ATR_pgs_67_67.pdf .