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CHAPTER 1

THE IMPORTANCE OF 17β-ESTRADIOL ON THE REPRODUCTION OF DECAPODS

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1. INTRODUCTION

Decapod crustaceans (crayfish, crabs, prawns, lobsters and shrimps) which include approximately 15000 species, are creatures of great interest in terms of production and cultivation due to their economic importance as well as their ecological importance in their environments. For this reason, the production and cultivation of decapod crustaceans is widely carried out on a global scale (Ikhwanuddin et al., 2019; Aaqillah-Amr et al., 2021; Albalat et al., 2022). Moreover, farming decapod crustaceans is a major economic driver in numerous countries, with yield attaining in 2018 approximately 10 million tonnes (US$70 billion). These productions are currently led by the cultivation of *Penaeus vannamei* (the Pacific whiteleg shrimp). About 170 billion farm-reared *P. vannamei* are produced each year (Albalat et al., 2022). Some other important farmed decapod crustaceans are:

Shrimps and prawns

- *Penaeus monodon* (the giant tiger prawn, Asian tiger shrimp, black tiger shrimp)
- *Penaeus japonicus* (the Kuruma prawn)
- *Macrobrachium rosenbergii* (the giant river prawn or giant freshwater prawn)
- *Fenneropenaeus indicus*, formerly *Penaeus indicus* (the Indian prawn)
- *Parapenaeus longirostris* (the deep-water rose shrimp)

Lobsters

- *Panulirus interruptus* (the California spiny lobster)
- *Panulirus ornatus* (the ornate lobster)
- *Panulirus homarus* (the Scalloped spiny lobster)
- *Homarus americanus* (The American lobster)
- *Homarus gammarus,* (the *European lobster or common lobster*)

Crabs

- *Scylla serrata* (the giant mud crab or the mangrove crab)
- *Scylla paramamosain* (the green mud crab)
- *Portunus pelagicus* (the blue swimmer crab)
- *Callinectes sapidus* (the blue crab)
- *Cancer pagurus* (the brown crab)

Crayfish

- *Procambarus clarkii* (the red swamp crayfish),
- *Cherax destructor* (the yabbie),
- *C. tenuimanus* (the marron),
- *C. quadricarinatus* (the red claw),
- *Astacus astacus* (the noble crayfish),
- *Pacifastacus leniusculus,* (the North American signal crayfish)

Latterly, monosex reproduction has been carried out for decapod crustaceans because males develop quicker and are considerably combative than females (Mohanakumaran Nair et al. 2006). Many of the investigations were intended on the producing males in decapods applying androjenic gland transplant or hormone utilization (such as: 17β-estradiol and 17a-methyltestosterone) to premature females (Baghel et al., 2004; Ohs et al., 2006). Yielding of unsterile neofemale (ZZ♀) is the first crucial period to reproduce all-male broods in decapods in which ZW is the gender assignment technic (Rungsin et al., 2012).

Natural or synthetic hormone applications have begun to be used in culture studies of aquatic creatures around the world, especially in the culture of economically important fish species, in order to increase breeding efficiency and to obtain female individuals, and very successful results have been achieved (Harlıoğlu and Farhadi, 2017; Lu et al., 2018; Jayasankar et al., 2020; Wang et al., 2022). For example, natural and synthetic hormones have been used to increase the productivity and produce single-sex populations of approximately 40 species belonging to the Salmonidae, Cichlidae, Cyprinidae, Anbantidae, Poecilidae, Cyprinodontidae and Ictaluridae families (Aktaş, 2006). The most important hormones used are; 17α-methyltestosterone (MT), trenbolone acetate (TA) and 17β-estradiol (E2). The use of these hormones, MT and TA, gave positive results in masculinizing fish fry, while the use of E2 gave positive results in feminizing them. On the other hand, compared to fish, the use of hormones in culture and sex change in crustaceans is at an early stage (Aktaş, 2006; Aktaş and Genç, 2011; Harlıoğlu et al. (2016, 2017a, 2017b, 2018).

2. THE IMPORTANCE OF 17β-ESTRADIOL ON THE REPRODUCTION OF DECAPODS

The 17β-estradiol (E2) is the fundamental female sex hormone take roles on the growth and care of female reproductive organs (Aktas and Genç, 2011; Harlıoğlu et al., 2017b; Bal and Harlıoğlu, 2021; Vanlı and Harlıoğlu, 2021). Most crustaceans have E2 naturaly to endocrine regulation of breeding (Mechoulam et al., 1984; Ryan 1982; Amin-Safwan, 2019). For instance, a

decisive rapport between vitellogenin amounts in hemolymph and circulatory volums of E2 was determined in shrimp *Penaeus monodon* by Quinitio et al. (1994); in prawn *Marsupenaeus japonicus* by Yano et al. (2000); and in crab *Mictyris brevidactylus* by Shih (1997). Moreover, amounts of E2 in the gonads and hemolymph at various vitellogenic periods of crab *Scylla serrata* have also been determined by Warrier et al. (2001). Similarly, Lafont and Mathieu (2007) reported that this hormone is found in the hepatopancreas, ovary and hemolymph of the crustacean, and its level varies according to the developmental stage of the ovaries. In addition, E2 caused vitellogenin synthesis in decapods (Subramoniam., 2011) and gonad growth in crayfish *Cherax albidus* (Coccia et al., 2010), and in *Penaeus japonicus* (Yano and Hoshino 2006). It was also found that E2 prompted lipogenic action in the gonads of prawn *Macrobrachium rosenbergii* (Ghosh and Ray, 1994). Latterly, E2 injections have been demonstrated to act a performance in the feminization of decapod crustaceans (Aktaş and Genç, 2011 and Harlıoğlu et al. (2016, 2017a, 2017b, 2018).

3. INCREASING PRODUCTION AND AQUACULTURE EFFICIENCY IN DECAPODS BY USING 17β-ESTRADIOL

3.1. **Shrimps and prawns:**

Nearly for two decades, gender manipulations has been performed in the farming of decapods. There are two common methods: (1) genetic and (2) endocrine administration (Devlin and Nagahama, 2002). Hormone applying is comparably effortless way to carry out and possess acceptable performance and cheap expenditure to obtain whole the uniform gender individuals in comparison to genetic manipulation (Falahatkar et al., 2014). Moreover, E2 is known to cause feminization in decapods (Wang et al., 2022). For example, Aktaş (2006) investigated the production of monosex, female green tiger shrimp (*Penaeus semisulcatus*) using E2 in his study.

The study by Aktaş (2006) consisted of two main experiments. In the first trial, the effects of E2 application in the form of bath on survival, feminization and growth rate of *P. semisulcatus* at different stages (egg, nauplii, protozoea, mysis, postlarva) were investigated. In the second attempt; The effects of feeding Artemia salina enriched with E2 as food during the mysis period on the survival, feminization and growth rates of shrimp were investigated. As a result of the study, Aktaş (2006) found that application of E2 in the form of a bath at a level of 50 µg/L significantly reduced the survival rate in the egg, naupli and protozoea stages. Among the periods treated with E2 at different life stages, the best feminization rate was found for the naupli group with 71.88%, and it was determined that this value was statistically different from the control group and other groups ($P<0.005$). In terms of growth and development, although the best average final live weight feminization rate was

obtained from the naupli group (14.15±2.41 g), the difference was found to be insignificant at the 5% significance level compared to the control group. In the second experiment, Aktaş (2006) found no statistically different effects on survival rate and feminization of shrimp fed with E2-enriched *A. salina* at the mysis stage compared to the control.

In Penaeus monodon, a marine shrimp, 12 steroids (Estrone, 17β-estradiol-Estriol, Androstenedione, Dehydroepiandrosterone, 5a-Dihydrotestosterone, Testosterone, Pregnelone, Progestrone, 17a-hydroxyprogestrone, 20a-Hydroxypregn-4-en-3-one) were detected in various stages of vitellonogenesis) was found and it was observed that the ovary development and maturation of this species was similar to that of fish (Fairs et al., 1990). Therefore, Fairs et al., (1990) argued that these estrogens may also play a role in determining gender in shrimps.

Pakdeenarong and Damrongphol (2006) investigated the effluences of E2 on the larvae and embryos of prawn, *M. rosenbergii*. Embryo samples of this species were maintained for 48 hours to 1, 10, or 50 μg/ml E2. At the end of the study there was no important differences in survival or in growth rate with the embryos exposed as controls, but the embryos maintained to 10 μg/ml E2 displayed a rise in hatching ratio, in primordial germ cell numbers, and in the ratio of incorporation of the primordial germ cell numbers into the growing gonads. In addition, new born larvae maintained to 1, 5, or 10 μg/ml E2 for 48 hours could not display meaningful differences in development rate and survival, in comparison to the controls. Nevertheless, larvae maintained to 5 or 10 μg/ml E2 displayed barely more enlarged gonads.

Choi et al (2020) studied the impacts of E2 injection and red light on the sexual development and vitellogenesis in shrimp *Lysmata amboinensis* by calculating the modify of mRNA expression status of eyestalk and vitellogenesis-related hormones induced by E2 injection and red light. They found that the E2 injection did not influence alterations of eyestalk hormones. Nevertheless, they also found that he red light gave rise to an importanrt enhance ($P < 0.05$) of crustacean hyperglycemic hormone encouraging adultness and a important diminish of vitellogenesis-inhibiting hormone that hiddened vitellogenesis. Therefore, Choi et al (2020) concluded that red-light irradiation can be utulised as a novel maturity-encouraging choice technique in the reproduction of, *L. amboinensis*.

The role of E2 and 17α-methyltestosterone (MT) on the development rate and ovarian growth of juvenile *M. nipponense* was researched by Jin et al. (2022). It was found that 500 mg/kg significantly encouraged the development of juvenile of female and male *M. nipponense* respectively ($P < 0.05$). On the other hand, E2 caused greater influences than MT on development performance. Moreover, the amounts of testosterone reduced with raised E2

dosages ($P < 0.05$) and smaller MT dosages (≤ 250 mg/kg) encouraged the realising of E2 ($P < 0.05$), although higher dosages caused an adverse influence ($P < 0.05$). Jin et al. (2022) concluded that 500 and 1000 mg/kg E2 and MT inclusion to diet approved the development and repressed gonadal growth in the juvenile of *M. nipponense*.

The effect of dietary inclusion with various amounts of E2 on inducing sex alteration in *Macrobrachium nipponense*, and preferred the genfer-revealed genes by achieving the ovarian transcriptome assay of naturally male *M. nipponense*, naturally female *M. nipponense* sex-altered male *M. nipponense* and unaltered male *M. nipponense* by Cai et al. (2023). The finding of Cai et al. (2023) showed that compared to the control, after approximately six weeks, consuming E2 with 200 mg/kg at PL25 caused to have the maximum gender proportion (female: male) of 2.22:1. Therefore, Cai et al. (2023) stated that dietary E2 leads to sex alteration obtaining important indication for setting up monoculture of *M. nipponense*. However, Cai et al. (2023) also reported that caution should be exercised to avoid disproportionate E2 inclusion in the diet.

3.2. **Crayfish**

Rodríguez et al. (2002a) in their study investigating the effects of some steroids on ovarian growth before vitellogenesis in *P. clarkii*, found that E2 and 17a-hydroxyprogesterone increased the gonadosomatic index to a statistically significant extent. Rodrguez et al. (2002b) also investigated the effects of methyl farnesoate alone and methyl farnesoate together with some hormones, including E2, on ovarian expansion before vitellogenesis in *P. clarkii*. At the end of the research, Rodríguez et al. (2002b) determined that the use of methyl farnesoate together with E2 positively affected oocyte (immature female gamete) growth in this species.

The change of E2 and 17α-hydroxyprogesterone levels in the hemolymph, hepatopancreas and ovary of wild-caught *A. leptodactylus* during oocyte development was investigated by Malati et al. (2013). Thirty individuals were used for this purpose. Individuals were kept in the laboratory at 25 °C under a 14-hour light-10-hour dark light regime between November and March. Findings showed that the E2 level in the hemolymph and ovary increased during vitellogenesis and declined in the hepatopancreas. In the same study, they found the maximum E2 level in the ovary (Malati et al., 2013). Additionally, Malati et al. (2013) as a result of their studies, E2 level (pg/ml); In the vitellogenesis cycle; They found 307 in the hemolymph, 700.8 in the ovary and 647.4 in the hepatopancreas, 182 in the blood, 1140.4 in the ovary and 880.6 in the hepatopancreas in individuals with mature ovaries, and 58 in the blood, 545.2 in the ovary and 620 in the hepatopancreas in individuals who produced pleopodal eggs.

Although there were many studies published on the mono-sex production of fish species and very limited number of studies published on that of crustaceans, and only a few studies have been published on the mono-sex production of freshwater crayfish up to date. Harlıoğlu et al (2016, 2017a, 2017b, 2018) investigated the possibility of producing 100% mono-sex individuals and increasing reproductive efficieny /(i.e., pleopodal egg number) of crayfish *Astacus leptodactylus* by use of E2 injections, In addition,,the levels of E2 in the ovary, hepatopancreas and heamolymph of this species after injections were also observed by Harlıoğlu et al (2016, 2017a, 2017b, 2018). For this reason, prior to the reproduction period adult *A. leptodactylus* samples caught from wild were placed into controlled concrete tanks and were fed with pellets prepeared to their nutritional requirements in order to allow them to adapt pellets and environmental conditions. After that E2 injections (10^{-7}mol/crayfish) were employed to the females ones in three weeks time period before their mating and pleopodal egg laying starts and E2 bath was applied to the pleopodal eggs and stage 1, 2 and 3 juveniles of E2 injected females. Bath applications (50 µg 17β-estradiol /L) were carried out to the pleopodal eggs monthly, and only one to the juveniles of each stage.

At the end of the study it was found that the E2 injections to the females gave rise to a significant enlargement in the reproductive efficiency, gonadosomatic and hepatosomatic index values in this species ($P < 0.05$). Hormone injection also created a significant raise in the levels of E2 in heamolymph, gonad and hepatopancreas in comparison to the control ($P < 0.05$) (Harlıoğlu et al., 2017b, 2018). Furthermore, the findings displayed that both E2 injection to the females and bath to the pleopodal eggs and juveniles gave rise to a significant increase in feminization in *A. leptodactylus* ($P < 0.05$). The lowest female rate (51.02%) was obtained from the control and the highest female rate (79.95%) was obtained from the females E2 injected and bath implemented for their both pleopodal eggs and juveniles (Harlıoğlu et al., 2017a).

3.3. **Lobster**

In a study, Couch et al. (1987) found that the E2 level in the hemolymph and tissues of the sea lobster *Homarus americanus* varied depending on the development status of the ovaries.

Nan et al. (2015) included steroid hormones to the diet of *P. interruptus*. They found that the inclusion of hormone to their diet caused a rise in the percentage of females having eggs (approximately 14%) and fertilized female number in comparison with the control. In addition, the period of carrying eggs from fertilization until hatching of the larvae was considerably shorter.

3.4. **Crabs**

The effects of E2 on survival percentage, ovarian growth, and definition degrees of genes practically associated with gonad growth in female *Portunus trituberculatus* were evaluated by Lu et al. (2018). They found that E2 injections seriously increased E2 concentrations in the hemolymph and encouraged the gonadal growth in *P. trituberculatus*. 1 μg E2 g–1 body weight injections caused the maximum gonadosomatic index ($P < 0.05$) in this species. On the other hand, there was not an important difference between survival and hepatosomatic index among all groups. Moreover, Lu et al. (2018) also found that E2 injections substantially promoted the gene expression amounts of ecdysone receptor-mRNA and retinoid X receptor-mRNA in the cerebral ganglion ($P > 0.05$), but depressed estrogen-related receptor-mRNA expression in the ganglion ($P > 0.05$). Therefore, Lu et al. (2018) concluded that E2 injections obliquely encourage the gonadal growth and vitellogenesis in the female of this species by mediating the releasing of hormones and gene expression in mandibular organ and eyestalk.

Liu et al. (2018) studied the effects of E2 (0, 10^{-10}, 10^{-9}, 10^{-8}, $10^{-7}$and 10^{-6} mol/L) on vitellogenin and vitellin amounts in the ovaries and hepatopancreas in crab *P. trituberculatus*. They were also further measured the oocyte diameters in this species. At the end of the study Liu et al. (2018) found that the amount of vitellogenin, the contents of vitellin and the diameters of oocyte were enormously developed in small dosages of E2 application (10^{-9}, 10^{-8} mol/L). Nonetheless, the amounts of vitellogenin in the hepatopancreas of *P. trituberculatus* were not increased by increasing dosages of E2. Therefore, Liu et al. (2018) concluded that E2 has a serious influence on vitellogenesis in the gonads during the vitellogenic period, however the influences of E2 are concentration-dependent and period-specific.

Dvoretsky et al. (2021) investigated the existence of sex hormones testosterone and 17β-estradiol in the hemolymph of crab *Paralithodes camtschaticus* using radioimmunoassay. They found that these parameters were 0.46 ± 0.04 ng mL^{-1}, and 1248.9±91.4 pg mL^{-1}, respectively in the hemolymph of this sğecies. In addition, they also found that the amount of testosterone and 17β-estradiol was not did not vary significantly between sex, and between mature and immature individuals of *P. camtschaticus* As a result of the study, it was evaluated that the development rate of *P. camtschaticus* was slow and the findings obtained would be useful both for further physiological research and for the improvement of reliable approach for the reproduction of *P. camtschaticus* (Dvoretsky et al., 2021).

4. CONCLUSION AND RECOMMENDATIONS

4.1. Conclusion

The effects of E2 in the reproduction of decapods are of predominant importance, governing many aspects such as gonadal growth, vitellogenesis and sex determination. Therefore, this study comprehensively considered the literature on the application of E2 in the culture of shrimps, prawns, crayfish, lobsters, and crabs, highlighting its potential in especially achieving monosex populations, enhancing reproductive efficiency, and influencing the growth of juveniles. It can also be concluded that E2, as a natural female sex hormone, governs the regulation of breeding processes in decapods. It has been linked to vitellogenin synthesis, gonad growth and feminization in numerous species, showcasing its versatility in influencing different reproductive aspects.

4.2. Recommendations

Further Research on Hormonal Manipulations: While this study provides valuable insights into the use of E2 in decapod culture, further research is recommended to explore additional hormonal manipulation techniques. Comparative studies with other hormones, as well as combinations of hormones, could shed light on more effective strategies for monosex production and enhanced reproductive efficiency.

Ecological Impacts: This study primarily focuses on the economic benefits of hormonal interventions in decapod culture. However, future research should also address the potential ecological impacts of hormone usage, considering the wider environmental implications and sustainability concerns associated with large-scale aquaculture practices in huge farms.

Species-Specific Studies: The diverse range of decapod species necessitates species-specific investigations to understand the variations in hormonal responses. Future studies should delve deeper into the intricacies of each species, considering their unique reproductive physiology and the optimal conditions for hormone application.

Long-Term Effects: The majority of studies discussed in this study evaluated short-term effects of E2 administration. It is also crucial to conduct long-term studies to assess the sustained impacts on reproductive performance, health, and overall well-being of decapod populations subjected to hormonal interventions.

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CHAPTER 2

RHODODENDRONS; HIDDEN WEALTH OF ECOSYSTEMS

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1. General Information

Rhododendron is a flowering species belonging to the Heather family and consists of woody shrubs. Many of these species do not grow very tall, so they can also be grown in pots. The scientific name of the *Rhododendron* is *Rhododendron*, a combination of the words "tree" and "rose". The *Rhododendron* tree is among the species that shed their leaves in winter. However, some species retain their green leaves in summer and winter. It has flowers in colors such as yellow, red, pink, purple and blue and is a very showy plant. The main reason for this is its abundant and colorful flowers. It blooms in summer and spends the months of June, July and August in flower. Although it is usually seen in shrub and dwarf tree form, it is also observed to be woody in places. It produces plenty of seeds and the seeds are quite small. *Rhododendron*, which has about 130 species, is common in mountainous areas with abundant rainfall in Turkey.

Rhododendrons are an important natural resource, especially in the Eastern Black Sea region, preventing soil from washing away on steep slopes. However, the challenges that these plants pose for forestry operations and their biomass value indicate that they should be utilized not only for erosion control but also as a source of renewable energy.

1.1. Distribution Areas and General Characteristics of *Rhododendrons* in Turkey

Rhododendrons, some species of which are very common in the Black Sea region, are popularly known as "komar" or "zifin" and some species are also used as fuel. *Rhododendrons* grow well in acid soils with high moisture content, rich in organic matter, deep and well drained. Average annual temperatures in these areas are generally above 13°C. On the Black Sea coast, average January temperatures are above 5°C in most places. In Sinop, Samsun and Trabzon, December averages approach 10°C (Sinop and Trabzon 9.4°C and Samsun 9.3°C). In the two hottest summer months (July and August), temperatures are above 20°C. Annual precipitation amounts are also quite high in the areas where *Rhododendrons* are distributed. As is known, especially in the eastern part of the Eastern Black Sea region, rainfall exceeds 2000 ml. However, the most important feature in terms of the distribution of precipitation over the seasons, as well as the amount of precipitation, is the high amount of summer precipitation falling on the Black Sea coasts. While summer precipitation rates reach 15% of the annual precipitation in most places, in some places it approaches 20% of the annual precipitation. It even exceeds 20% of the annual precipitation in Rize (20.3%). This characteristic in summer precipitation rates generally continues from the Black Sea coasts to the inland regions. For example, in Artvin and Gümüşhane, although the annual precipitation is significantly less than the coastal stations, the summer precipitation rate is over 15% (Avcı, 2004) (Figure 1-2).

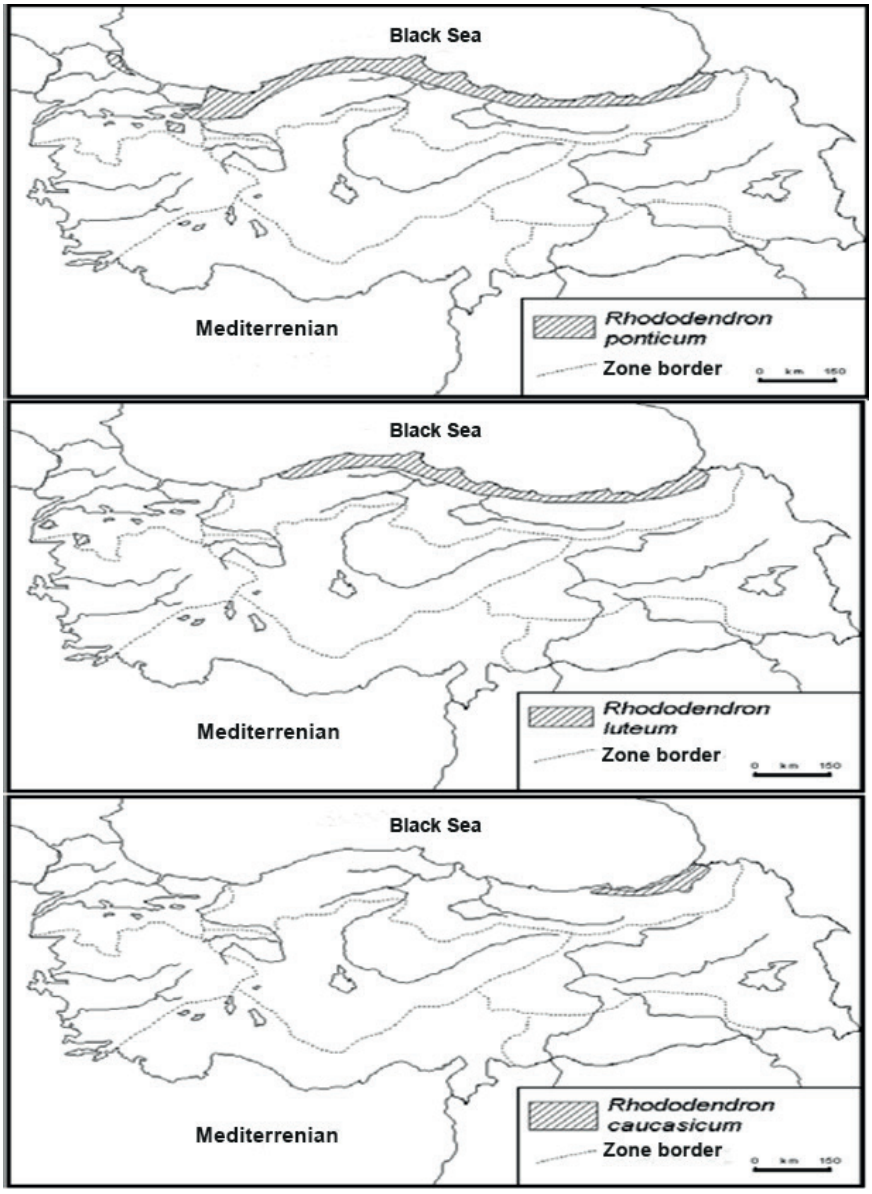

Figure 1. Distribution areas of *Rhododendrons* in Turkey

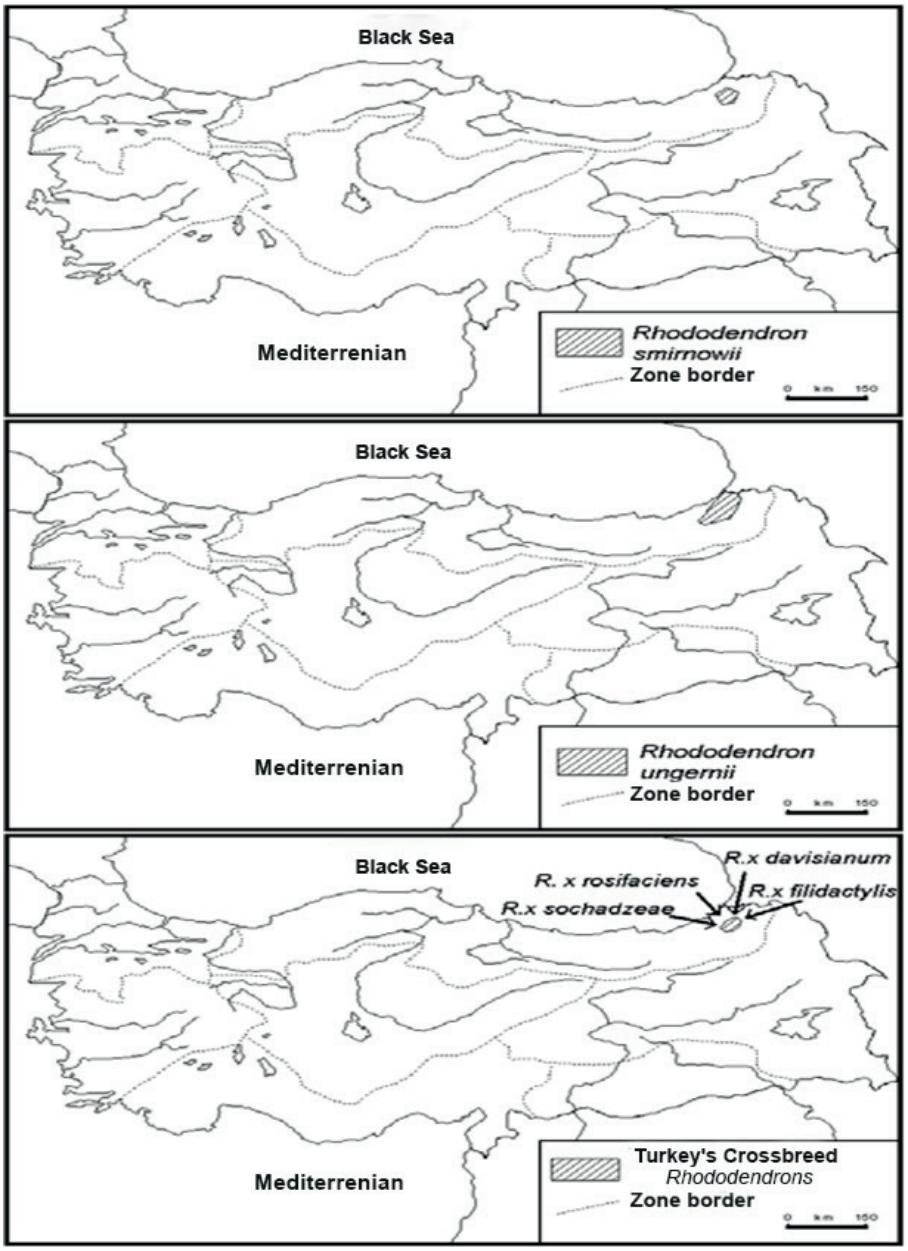

Figure 2. Distribution areas of *Rhododendrons* in Turkey

2. *Rhododendrons* Growing Naturally in Turkey

2.1. Purple Flowering *Rhododendron* (*Rhododendron ponticum*)

It grows on all coasts of the Black Sea. It is a shrubby plant with dense branches. Since its leaves do not fall off in winter, it is one of the evergreen species. It is defined as a shrub or small tree species that blooms in spring

and maintains this flowering state for a long time, and can reach up to 8-10 meters in good growing conditions, called "kara kumar/komar", "Kara Ağu" or "kumar" in the Black Sea region. It is one of the best known species of *Rhododendrons*. *Rhododendron ponticum* is distributed along the northern slopes of the mountains in Turkey, starting from the Istranca Mountains in the west to the eastern Black Sea coastal mountains in the east. In the Black Sea region, it is also used as fuel in some areas and charcoal is produced in some places. The other three species of the section are distributed in North America. The scientific discovery of *Rhododendron ponticum*, one of the seven *Rhododendron* species naturally distributed in Europe, goes back a long way. It is among the six *Rhododendrons* described by Linnaeus in 1753. Its 12-15 cm long leaves are bright green. The purplish pink flowers are 5-20 in number. These flowers may rarely be white in individuals of *R. ponticum*, a natural form of *R. ponticum*. It is usually the dominant *Rhododendron* in beech forests. Its vertical distribution is between sea level and 1800 meters in most places, but it rarely reaches elevations of 2000 meters. As the habitat conditions become more difficult as the elevation increases, the leaf size decreases and it becomes a shrub of 1-2 m in height (Avcı, 2004) (Figure 3).

Figure 3. ***Rhododendron ponticum***

2.2. Yellow Flowering *Rhododendron* (*Rhododendron luteum*)

It grows in the Black Sea, central east and west. *R. luteum*, popularly known as "Eğri flower", "çifin", or "Sarı Ağu", is a shrub species that sheds its leaves in winter, unlike other *Rhododendron* species known to be distributed in Turkey. *R. luteum* is the only deciduous *Rhododendron* species in Europe and Southwest Asia. It can grow up to about 4 meters tall and 5-15 of its yellow flowers are found together at the shoot tip. *R. luteum*, with a vertical range of 400-2000 meters, is a *Rhododendron* that rarely reaches areas close to the tree line. The yellow flowered *Rhododendron* was collected and described by Tournefort from the Trabzon region in the 1700s. Yellow-flowered *Rhododendron* has a wide distribution area in the Black Sea region and extends westward to Balıkesir and Çanakkale (Avcı, 2004) (Figure 4).

Figure 4. ***Rhododendron luteum***

2.3. White Flowering *Rhododendron* (*Rhododendron ungernii*)

It grows only in the Artvin region. Also known as "White Kumar / Komar" in its natural distribution area in Turkey, it is an evergreen *Rhododendron* that grows about 6-7 meters tall. There are flower clusters consisting of 12-24 flowers on the flower stalk. *R. ungernii* with dull pinkish-white flowers is an Euxine flora element (Figure 6). Its distribution area is similar to *R. smirnowii*, but it ranges a little further north in Adzhariya in the Caucasus Mountains. This species is also mesophyllous, occurring mostly on north and east facing slopes of mountainous areas. It is distributed in beech, spruce or mixed forests. It forms mixed communities with *R. smirnowii*, especially in the belt between *R. ponticum* and *R. caucasicum*. Its vertical distribution ranges between 800-2000 meters, reaching up to 2200 meters in some areas. In the Eastern Black Sea region of Turkey, where the Colchic area is represented, it has a distribution area especially near the border with Batumi, Murgul Şavval Hill, Tiryal Mountain and Kaçkar Mountains.

As in the distribution areas of *R. ponticum* and *R. luteum*, which are the most common species in Turkey, many *Rhododendron* areas are transformed into *Rhododendron* control areas in various ways due to its harmful effect on the rejuvenation of other forest trees. *R. ponticum*, known by foresters as "stubborn sapwood", is an important cover plant. Vegetative debris in the area where it is found often cannot decompose. For this reason, it both prevents the rejuvenation of other plants and impoverishes the soil fauna. The fight against *Rhododendrons* is not only in our country, but also in different parts of the world. Similar methods of control are also used in these areas. It is also known that a great deal of research has been carried out, especially in the UK, on the control of *Rhododendron* by chemical methods. In the Black Sea region, new methods of controlling *Rhododendrons* have been the subject of researches due to the fact that they significantly hinder rejuvenation efforts in beech and spruce forests (Avcı, 2004) (Figure 5).

Figure 5. ***Rhododendron ungernii***

2.4. Red Flowering *Rhododendron* (*Rhododendron smirnowii*)

It is found in Rize and Artvin provinces. *R. simirnowii*, called "Red Kumar" in the Eastern Black Sea region, was discovered by Baron Ungern Sternberg around Artvin in 1885, described by Trautvetter in the same year and named after M. Smirnov, Sternberg's friend.

Its distribution area in the Eucine flora is very limited. It was known as an Anatolian endemic for many years. However, when it was found in the southwest of the Caucasus Mountains in 1962, the information about its distribution area changed. *R. smirnowii*, an evergreen *Rhododendron* species, grows about 4 meters tall. The underside of the dark green leaves and the shoots bearing the flowers are covered with dense gray-white hairs. The bright pink flowers have 7-15 of them together (Figure 5). Its vertical distribution range varies between 850-2300 meters in Anatolia, but it is mostly concentrated between 1600-2200 meters. In beech forests at 1000-1600 meters in the Caucasus Mountains, it forms mixed communities with *Rhododendron ponticum* and *R. ungernii* or alone. In northeastern Anatolia, *R. smirnowii*, which finds habitat in spruce and fir forests on volcanic areas or on soils with limestone bedrock, was cultivated one year after its discovery (Avcı, 2004) (Figure 6).

Figure 6. ***Rhododendron smirnowii***

2.5. Caucasian *Rhododendron* (*Rhododendron caucasicum*)

It is found in the Black Sea regions of Rize, Trabzon and Artvin. The tree, which is up to 1 meter tall, is always green and its flowers smell good. It is also found in Erzurum and Kars provinces. The name of the Caucasian *Rhododendron* in the Eastern Black Sea Region is "Mountain Sandal". Its white or cream colored flowers are eaten by the people. This *Rhododendron*, which can grow up to 1 or 1.5 meters tall, is found on the acidic soils (pH 3-4) of the Black Sea region, especially on the northern slopes of mountainous areas. Although it forms scattered communities in the understorey of beech, beech-fir or beech-ladin forests, it is stated that the best growing conditions are in the subalpine belt and alpine belt. For this reason, Caucasian *Rhododendron* is also described as a high mountain species (Figure 4). It has a wide distribution area in the Caucasus Mountains, from the northwest of the Greater Caucasus Mountains to the west of Azerbaijan and upstream of the Samur River in Dagestan. Following its distribution in the Lesser Caucasus Mountains, it is connected to its distribution area in the Northern Anatolian Mountains. *R. caucasicum*, which grows on the north-facing slopes of the North Anatolian Mountains on soils with high humidity and sometimes peat character, extends as far west as the Soğanlı Pass between Trabzon and Bayburt. Ferik Mountain, Tiryal Mountain, Kürdevan Mountain and Yalnızçam Mountains are among the other mountainous areas where Caucasian *Rhododendron* is distributed in North Eastern Anatolia. Around Gül Mountain southwest of Murgul, it forms a community at 2300-2400 meters together with Daphne glomerata at Şavval Hill. The shoots of this *Rhododendron* with dull cream-colored flowers and 5-8 flower clusters at the shoot tips are also slightly hairy. Although the vertical distribution range of *R. caucasicum* is generally between 1800-3000 meters, it grows best at elevations above 2000 meters. Caucasian *Rhododendron* can reach up to 3,250 meters in the Kaçkar Mountains. Caucasian *Rhododendron* blooms 3-4 weeks earlier than other species, which increases its value as an ornamental plant (Avcı, 2004) (Figure 7).

Figure 7. *Rhododendron caucasicum*

3.Diversity and Distribution of *Rhododendrons*

Rhododendrons are distributed in the Black Sea region in a special climatic condition characterized by high rainfall and hot summers. These plants play a critical role in the ecosystem balance of the region, especially in provinces such as Artvin and Gümüşhane, with summer rainfall accounting for a significant proportion of the annual precipitation.

The Black Sea region is home to various species of *Rhododendron*. The most notable of these species are the yellow-flowered *Rhododendron* (*Rhododendron lut*eum) and the purple-flowered *Rhododendron* (*Rhododendron ponticum*). The yellow-flowered *Rhododendron* is a shrub that sheds its leaves in winter and can grow up to about 4 meters in height. This species has a wide distribution in the Black Sea region and is especially dense around Trabzon. The purple-flowered *Rhododendron,* on the other hand, is an important species for the understory flora in Artvin and its surroundings.

3.1. *Rhododendron* Species and Distribution Areas in Artvin

In a preliminary study conducted by the Artvin Regional Directorate of Forestry, all areas were surveyed and the existing *Rhododendron* species, their distribution areas and quantities were determined. The estimated areas where *Rhododendron* is very dense are shown below (Table 1). The total of these areas is around 39601 Ha. This area corresponds to approximately 1/10 of the total forest area of Artvin Province (Özkaya, 2016).

Table 1. Intensive *Rhododendron* areas of Artvin Regional Directorate of Forestry

Management Directorate	Management Directorate Management	Location	Area (Ha)	*Rhododendron* species
Artvin	Artvin	Belalıkaya	200	*R. ponticum*
		Mersivan	150	*R. ponticum*
	Zeytinlik	Yayladere	285	*R. luteum-R. ponticum*
		Ortayol	64	*R. luteum-R. ponticum*
		Kömürlüdere	79	*R. ponticum*
	Atila	Kışladere	150	*R. luteum*
		Soçidibi	200	*R. smirnowii*
		Danayayımı	80	*R.luteum*
	Tütüncüler	Forest area	5500	*R. ponticum*
		Over the Çoruh	1000	*R. luteum*
		Plateau the Ormanlı	10	*R. caucasicum*
		Plateau the Ormanlı	10	*R. smirnowii*
	Saçinka	Kazankaya	80	*R. luteum-R. ponticum*
		Karagöl	40	*R. luteum-R. ponticum*
		Buharet	15	*R. luteum-R. ponticum*
		Beşağıl	75	*R. luteum-R. ponticum*
		Erenler	50	*R. luteum-R. ponticum*
		Avcı Kilise	55	*R. luteum-R. ponticum*
		İnekdağı	20	*R. luteum-R. ponticum*
	Ortaköy	Çakmaklı	5	*R. ponticum*
	Taşlıca	Golahora	2000	*R. ponticum*
		Çitimzara	1500	*R. luteum*
		Fındıklıdere	1500	*R. caucasicum*
		Çayırlık	500	*R. ponticum*
Arhavi	Arhavi	Konaklı	165	*R. ponticum*
		Şenköy	150	*R. ponticum*
		Plateau the Acıgöl	667	*R. ponticum*
	Arhavi	Dülgerli	227	*R. ponticum*
		Boyuncuk	350	*R. ponticum*
		Ulukent	150	*R. ponticum*
		Balıklı	325	*R. ponticum*
	Hopa	Yeşilköy	60	*R. ponticum*
		Balıkköy	308	*R. ponticum*
		Subaşı-Eşmekaya	340	*R. ponticum*
		Cankurtaran	38	*R. ponticum*
		Sultanselim	494	*R. ponticum*
		Esenkıyı	527	*R. ponticum*
Arhavi	Kayadibi	Armoni	50	*R. ponticum*
	Kayadibi	Kireçlik	50	*R. ponticum*
		Löme	400	*R. ponticum*
		Arılı highland road	400	*R. ponticum-R. caucasicum*
		Kamilet	150	*R. ponticum*
		Küçükköy	100	*R. ponticum*
	Kemalpaşa	Çamurlu	329	*R. ponticum*
		Köprücü	604	*R. ponticum*
		Osmaniye	158	*R. ponticum*

Borçka	Borçka	Zorlu Köyü	350	*R. luteum-R. ponticum*
		Taraklı	50	*R. ponticum*
	Balcı	Balcı	500	*R. luteum*
		Balcı	100	*R. ponticum*
	Camili	Camili	14370	*R.ponticum*
	Başköy	Akarşen	200	*R. ponticum*
	Karadağ	Karadağ	440	*R. ponticum*
		Karadağ	558	*R. luteum*
	Çifteköprü	Çifteköprü	134	*R. ponticum*
Borçka		Azure-Sümercalı	170	*R. ponticum*
	Göktaş	Plateau the Kokolet	80	*R. caucasicum*
		Köyiçi	20	*R. luteum*
		Lebüskür-Akarşen	40	*R. smirnowii*
		Kokolet-Akantaş	30	*R. ponticum*
	Kabaca	Telepınar	100	*R. ponticum*
		Köpürten	100	*R. ponticum*
		Fidanlık	40	*R. ponticum*
		İskale	60	*R. ponticum*
	Karşıköy	Karşıköy	700	*R. ponticum*
		Karşıköy	100	*R. luteum*
		Atanoğlu	1300	*R. ponticum*
		Atanoğlu	200	*R. ponticum*
	Karşıköy	Çaylı	100	*R. ponticum*
		Güreşen	100	*R. ponticum*
Şavşat	Meydancık	Papart	50	*R. caucasicum, luteum*
Yusufeli	Altıparmak	Altıparmak	25	*R. smirnowii*
		Altıparmak	20	*R. luteum*
		Altıparmak	12	*R. caucasicum*
	Öğdem	Demirköy-Ilgıt	22	*R. smirnowii*
		Demirköy-Nizgut	20	*R. luteum*

4. Importance of *Rhododendrons* for Forestry

They also have a significant potential in terms of biomass value and prevent soil washing and sliding on steep slopes. Research shows that the above-ground biomass value of these plants is quite high and should be considered as carbon sinks. *Rhododendrons* are also important as a sustainable source for bioenergy production. Nevertheless, *Rhododendrons* are an important problem for all forestry activities. As is known, information on *Rhododendrons* in management plans is mentioned in the living cover section. However, it is not possible to draw reliable results from these plans regarding the area and wealth inventory. An inventory study should be carried out to determine the exact distribution areas and to know the wood wealth. As a matter of fact, the General Directorate of Forestry commissioned a management committee in 1976 to carry out such a study in 6 series of Artvin Regional Forest Directorate. According to this study, the average *Rhododendron* wealth per hectare is around 450 pounds and 70% of the *Rhododendrons* have a center diameter of less than 10 cm (Karaçalıoğlu, 1974).

4.1. Benefits of *Rhododendron* when used in Renewable Energy Production

Turkey's biomass capacity, which is largely left to rot after the annual harvest, offers a great potential for energy production. In particular, regions covered with forests, such as Artvin, are highly suitable for biomass power plants. Establishment of these power plants,

- To make a sustainable contribution to overcoming the country's energy shortage by providing bioenergy production

- Eliminating ecological damage caused by *Rhododendron* to forest ecosystems, such as soil acidification, reduced biological activity and slowing of litter decomposition,

- Providing alternative employment and income sources for forest villagers,

- Provide employment and increase national income,

- Providing a positive contribution to wildlife and plant species diversity by removing *Rhododendron* under forest cover,

- It will be easier to rejuvenate forests naturally and rejuvenation costs will be reduced,

- By removing *Rhododendron* from the understory flora, the annual increment of forests is positively affected and the production per unit area increases.

4.2. Benefits of Using *Rhododendron* as Biofuel

Rhododendrons are a fast-growing plant that grows in mountainous regions with abundant rainfall in our country and gives seeds in a short time. The oil obtained from the seeds of this plant has attracted attention in recent years with its use as biofuel. There are many benefits of using *Rhododendron* oil as biofuel.

- Improvement in the socio-economic structure of rural areas and local job opportunities

- Contribution to preventing migration

- Creating new job opportunities

- Economic and strategic contribution to the country by reducing dependence on foreign oil

- Contribution to sustainable future and public health due to cleaner combustion products

- Protection of natural energy resources and the environment

- Support for sustainable energy

- Alternative to limited and exhaustible energy sources

- Reduced emission of harmful greenhouse gases (CO emissions are reduced by 50% when using biodiesel).

- Reduced air pollution and public health risks

- Extends the life of the engine.

- Anti - toxic, sulfur free.

4.3. Possibilities of Utilization of *Rhododendron* Wood

In the Black Sea coastal region where *Rhododendrons* are found, these woods are used as fuel and in some regions charcoal is produced. As can be seen in Table 2 below, it shows the tree species that are wood but have heat value.

Another area of utilization of *Rhododendron* wood is button making and vegetable knitting. Cellulose and alpha cellulose values of this wood are high. In this respect, it is thought to be an important raw material for the artificial silk industry.

Table 2. Caloric Values of Various Wood Types in Complete Dry State

Tree Species	Heat Value (Kcal/kg)
Abiete	5066
Fagus	4802
Abiegnis	4726
Abies	4651
Birch	4506
Oak	4356
Acer	4183
Poplar	4129
Hornbeam	4062
Lignin	6100
Cellulosum	3450
Resinae	8500
Rhododendron	**4667**

Rhododendron wood is also used in the fiberboard industry. The first application in this field was applied in Artvin Fiberboard Factory. However, production was terminated with the closure of the factory. Due to the limited forest resources in Turkey, if *Rhododendron* wood, which is an important source of fibrous raw material, is used in the production of medium density fiberboard (MDF), it will support the raw material shortage of this sector.

Rhododendron is a suitable raw material for MDF production both in pure and mixed form, and the boards produced have the values stipulated by the standards. *Rhododendron* shows a complete similarity to leafy trees

in terms of fiber properties and chemical composition. It can be used as an alternative raw material in areas where short-fibered coniferous trees are used in paper production. On the other hand, it is thought that it can be evaluated by blending with long-fiber coniferous trees, which will also provide a good surface smoothness.

Since *Rhododendron* has a high density, it can be used as an alternative raw material in areas related to forest products where high density coniferous trees are used. *Rhododendron* can also be utilized in particle board production due to its great raw material potential (Çamlibel, 2006).

5. Biomass Values of *Rhododendrons*

In a research area with an average slope of 38% and an average elevation of 1725 meters, the above-ground biomass value of *Rhododendron* was determined as 16058.68 kg/Ha and the total biomass value as 24457.12 kg/Ha. The leaf biomass of *Rhododendron* was 4349.36 kg/Ha and stem biomass was 11709.32 kg/Ha.

The average height of *Rhododendron* in the research area was 106.34 cm, the average density in the area was 78.15% and the average number of roots was 97312.30. The average fine root biomass value for *Rhododendron* in the research area was 4311,96 kg/Ha, the average thick root biomass was 4086,47 kg/Ha and the total root biomass value was determined as 8398,43 kg/Ha.

The average fine spring biomass value for *Rhododendron* in the research area was 3861.75 kg/Ha, the average thick spring biomass value was 2540.68 kg/Ha and the average spring total biomass value was determined as 6402.43 kg/Ha. In the research area, the average fine fall biomass value for *Rhododendron* was 4766.54 kg/Ha, the average coarse fall biomass value was 5630.44 kg/Ha and the average fall total biomass value was determined as 10402.95 kg/Ha (Özkaya, 2016).

6. Above Ground Values of *Rhododendrons*

Total biomass, above-ground biomass, below-ground biomass, stem and leaf biomass values of purple-flowered *Rhododendron* (*Rhododendron ponticum*) in Artvin province of the Eastern Black Sea Region were determined by researches. In order to determine the total biomass, below-ground biomass, above-ground biomass, stem and leaf biomass values of purple-flowered *Rhododendron* in a research area, experimental areas were selected according to the random sampling method. A total of 65 5x5 m research plots were selected in the whole research area (Figure 7). Root sampling was carried out by driving a 6.4 cm diameter steel cylinder with a depth of 35 cm into the soil and taking 5 root samples from each experimental area. For this purpose, a total of 325 root samples were taken from 65 experimental areas in spring and fall periods. Biomass regression equations of purple-flowered *Rhododendron* depending on height and degree of cover were formed by utilizing the biomass values obtained. The average above-ground biomass value of purple-flowered *Rhododendron* in the research area was 16.06 tons/Ha and the total biomass

value was 24.46 tons/Ha. The leaf biomass of purple flowering *Rhododendron* was 4.35 tons/Ha and stem biomass was 11.71 tons/Ha. The average above-ground biomass value of purple flowering *Rhododendron* constitutes 65.66% of the total biomass. Leaf biomass accounts for 27.08% of above-ground biomass and stem biomass accounts for 72.92%. It was determined that 17.78% of the total biomass consisted of leaf biomass and 47.88% of stem biomass (Özkaya, 2016) (Figure 7).

Figure 7. 5x5 m sample area taken from the study areas

7. Subsoil Values of *Rhododendrons*

According to the researches, the average fine root biomass value for Rhododendron was 4311.96 kg/Ha, the average thick root biomass was 4086.47 kg/Ha and the total biomass value was determined as 8398.43 kg/Ha. The average subsoil biomass value of *Rhododendron* constitutes 34.34% of the total biomass, fine root biomass constitutes 51.34% of the subsoil mass and thick root biomass constitutes 48.66%. Therefore, 17.63% of the total biomass is composed of fine root biomass and 16.71% of the total biomass is composed of thick root biomass.

As a suggestion; the amount of carbon stored in above-ground biomass in Turkey's productive forest areas is 31.41 t/Ha in coniferous forests, 53.30 t/Ha in broadleaf forests, 13.72 t/Ha in coppices and the average amount of carbon stored in productive forest areas is 32.44 t/Ha (Tolunay, 2011). A comparison of our data with the amount of carbon stored in the above-ground biomass in productive forest areas of Turkey shows that purple-flowered *Rhododendrons*, which constitute a significant part of the understory in our study area, have a carbon storage potential very close to the amount of carbon stored in coppices, and when compared with productive forest areas, their share in total biomass is considerably high. Nevertheless, it is evaluated that *Rhododendron* and similar shrubs and sapwood plant formations, which constitute the lower cover of forest vegetation in Turkey, have a much higher biomass value than estimated and that these species should also be given importance in their evaluation as carbon sinks (Özkaya, 2016).

8. Organic Compounds and Biological Activities of *Rhododendron* Flower

Research *on Rhododendron* species growing in Turkey has been increasing especially in the last 10-15 years and includes essential oils, diterpenic compounds, flavonoids and other phenolic compounds and biological activities of *Rhododendrons*. In the studies, as a result of the isolation of organic compounds from the flowers of the purple-flowered *Rhododendron* plant, which is an endemic species, and the investigation of their antioxidant activities, a triterpene, a steroid and several essential oils of undetermined structure were obtained from hexane and methylene chloride extractions of these plants. It was found that the steroid was β-sitosterol and the triterpene was ursolic acid. Spectral methods were used for the structure determination of the compounds. For this purpose, IR spectra were taken and single and double dimensional NMR (1H, 13C, BB, APT and DEPT) techniques were used. Known compounds were compared with standards by thin layer chromatography and melting point determinations were made. As a result of activity studies (DPPH Free Radical Scavenging Activity, β-carotene Linoleic Acid (Lipid Peroxidation Inhibition Activity) on various extracts of purple flowering *Rhododendron*, it was determined that these extracts had low activity. The idea that β-sitosterol obtained as a result of isolation, both alone and in combination with other similar phytosterols, reduces cholesterol levels in the blood and is sometimes used in the treatment of hypercholesterolemia and that sitosterol is useful in the treatment of prostate enlargement has gained certainty. In European countries β-sitosterol is used in the treatment of enlarged prostate (benign prostatic hypertrophy). Ursolic acid shows antimicrobial, antitumor, anti-inflammatory, antibacterial, antifungal and anti-HIV properties. Thanks to the results of these and similar studies, the export of medicinal and aromatic plants in our country to foreign countries can provide income to the local people and our country. In addition, determining the medicinal and aromatic potential produced from forest resources in recent years and the possibilities of valorization can provide many economic, social and environmental benefits (Işık, 2012).

9. Conclusion and Recommendations

Rhododendrons are an important natural resource that can be utilized in a wide range of areas, from ecosystem conservation to bioenergy production. Sustainable management and utilization of these plants will provide significant environmental and economic benefits.

The amount of *Rhododenron ponticum* (Purple Flowering *Rhododendron*), which causes significant problems especially in Artvin forests in rejuvenation areas, was determined in a certain basin in Çifteköprü and Karadağ Forest Management Supervisions of Borçka Forest Management Directorate and in a basin in Göktaş Forest Management Supervision of Murgul Forest Management Directorate by taking 5x5 test areas with random sampling method in forest areas with 1st, 2nd and 3rd degree closure at an average altitude of 1,000 meters per hectare. *Rhododendron* was cut 30 years ago in

Çifteköprü and Göktaş chiefdoms.

As a result; this important wealth, which is extracted from forests within the framework of a specific plan without harming the ecosystem of the forests, should be utilized not only as fuel, but also in the above-mentioned areas or in different areas. On the other hand, the removal of *Rhododendron* from forests will also contribute to the employment of forest villagers and this will reduce the intervention in forests a little more.

In our country, the state forests have a biomass capacity of 7 million m^3 of branches, bark, end pieces, stumps, stumps and roots, which are largely left to decompose after harvesting every year. It is calculated that it can produce energy equivalent to 6 million tons of oil per year. Unfortunately, there are no biomass power plants in Turkey to generate electricity and heat. Our province of Artvin, 55% of which is covered with forests, and the Black Sea Region, which has the same characteristics, are in a very suitable position in terms of biomass power plants suitable for the energy forestry project. In our region, which is covered with abundant forests and *Rhododendron*, the area in question should be utilized in modern energy forestry within appropriate planning in the areas of *Rhododenron* that are not produced, and the cutting of valuable trees as fuel should be prevented. In the biomass power plant to be established, electricity and heat can be produced by burning. While the forest villagers will earn income in return for the amount of wood they bring to the power plant, they will contribute to the regional economy. *Rhododenron* sapwood can also be used to make wood pallets together with other sapwood residues.

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CHAPTER 3

GRAPES AS AN IMPORTANT SOURCE OF ANTHOCYANINS

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1. INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the earliest domesticated fruits, also it is a fruit with high economic value due to different consumption patterns such as wine, table grape, raisins, vinegar, molasses, etc. (Myles et al., 2011; Alston and Sambucci, 2019; Khan et al., 2020). *Vitis vinifera* L. belongs to the family Vitaceae, which comprises about sixty inter-fertile wild *Vitis* genus (Terral et al., 2010; Myles et al., 2011). Other important grape cultivars belong to *V. labrusca, V. rotundifolia, V. rupestris, V. coignetiae, V. amurensis* species and their hybrids with *V. vinifera* or with each other (Allewerdt and Possingham, 1988; He et al., 2010). There are about 30.000 named grape varieties in the world, but it is thought that 15.000 of them may be genotypically different (Allewerdt and Possingham, 1988). According to 2021 data, world grape production is 78.034.332 tons in an area of 6.950.930 hectares (FAO, 2022).

Grapes are an important fruit because they are high in antioxidants as well as nutrients. The name anthocyanin comes from the Greek words ánthos: flower and cyanin: blue (Rhizopoulou, 2004). Anthocyanins are the largest group of phenolic pigments, proposed by the German pharmacist L. C. Marquart in 1835 to name the blue pigments of flowers. Today, not only blue, but also virtually all the blue, violet and red hues of flowers, stems, fruits, leaves and roots correspond to pigments of this kind (Bueno et al., 2012; Tena and Asuero, 2020).

Anthocyanin carries a positive charge on the oxygen atom of the C ring in the basic flavonoid structure, but is still considered one of the flavonoids (Husain et al., 2021; Gonçalves et al., 2021). Anthocyanin is one of the subclasses of phenolic phytochemicals. The best-known anthocyanins are cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside (Gonçalves et al., 2021). An anthocyanidin is an aglycone moiety that is formed by hydrolysis of anthocyanin glycoside (Jokioja et al., 2021). Presence of flavylium ion and its unusual electron distribution makes anthocyanidins highly unstable; therefore, the aglycone form of anthocyanins is extremely rare in nature (He et al., 2010). Conjugated bonds of anthocyanins result in red, blue and purple coloured plants. The stability of anthocyanin colour based on light, pH, temperature, and its structure. In acidic condition, anthocyanin appears as red pigment, while in alkaline conditions, blue pigment anthocyanin is present (Khoo et al., 2017).

Especially in recent years, anthocyanins are important due to their positive effects on human health. Anthocyanins have anticancer, antidiabetic, anti-inflammatory and anti-obesity effects, as well as being effective in preventing cardiovascular and neurodegenerative diseases etc. (Khoo et al., 2017; Dharmawansa et al., 2020; Mattioli et al., 2020). Furthermore, recent evidence suggests that anthocyanins may also be related to modulation of

gut microbiota (Mattioli et al., 2020). Therefore, anthocyanins obtained from edible plants are potential pharmaceutical ingredients (Khoo et al., 2017). Anthocyanins extracted from plants have been used as food additives. E163, the common name for E163 is the anthocyanins, is a food additive approved by the European Union (EU) (EFSA, 2013). The average daily intake of anthocyanins is estimated to be more than a few milligrams. Nutrition, gender, and the presence or absence of food intolerance affect the amount of anthocyanin intake in individuals (Pojer et al., 2013). Food consumption habits that vary by country have an impact on the amount of anthocyanin consumed daily. For example, the intake amount in the Netherlands, USA and Australia is estimated about 19.8, 12.5 and 24.2 mg per day per person, respectively (Wu et al., 2006; Gonçalves et al., 2021). In fact, the acceptable daily intake of anthocyanins has not been established (EFSA, 2013). However, food colours can be obtained naturally or synthetically. Due to their potential dangers, synthetic food colorants are being replaced by those obtained from natural sources (Silva et al., 2022).

2. ANTHOCYAN CONTENT OF SOME PLANTS

Anthocyanins are found in edible flowers, vegetables, fruits, some grains, seeds and leaves that have red, orange, purple and blue colours and their products such as fruit juices, tea and red wines (Khoo et al., 2017; Tena and Asuero, 2020; Gonçalves et al., 2021). The amount of anthocyanin varies depending on plant species and varieties (Table 1).

Table 1. Concentration of anthocyanins in some plants (mgC3G/100 g in fresh weight)

Plants	**Amount anthocyanin**	**References**
Açai	303	Rosso et al., 2008
Apple	26,8	Wolfe et al., 2003
Bilberries	300-698	Gizzi et al., 2016
Blackberries	201	Fan Chiang and Wrolstad, 2005
Blueberries	407	Cereze et al., 2020
Eggplant	129	Nayanathara et al., 2016
Grape	280-655	He et al., 2010
Pomegranate	490-1500	Karaaslan and İzol, 2014 Zhu et al., 2015
Red onion	20	Samir et al., 2019
Strawberry	60	Silva et al., 2007
Sweet cherry	300	Antognoni et al., 2020
Sour cherry	633	Damar, 2010
Tomato	7-283	Blando et al., 2019 Butelli et al., 2008

Additionally, it varies depending on cultural practices, ecology, climatic conditions, harvesting and storage conditions and processing conditions of the product (García-Beneytez et al., 2002; Gonçalves et al., 2021; Lago et al., 2023).

3. ANTHOCYANINS IN GRAPES

Anthocyanins are a class of important phenolic compounds that are primarily responsible for the red colour of grapes and wines (He et al., 2010). They are often seen on grape skins and their content increases until ripeness (García-Beneytez et al., 2002). However, grapes called "teinturier" contain anthocyanins in both their skin and flesh (Santiago et al., 2008; Uzun and Aktürk, 2019). Moreover, anthocyanin coloration was visually observed in cotyledon leaves of teinturier grape genotypes (Uzun and Aktürk, 2019).

There are many studies on anthocyanins found in grapes and grape-processed foods and their effects on human health (García-Beneytez et al., 2002; He et al., 2010; Khoo et al., 2017; Lago et al., 2023). Anthocyanins, which are formed by binding sugars to the anthocyanidin molecule, are named by specifying the name of the bound sugar and the position to which it is attached. For example; Anthocyanin, formed by binding a glucose molecule to the 3rd position of cyanidin; It is cyanidin-3-glucoside. For example; Anthocyanin, formed by binding a glucose molecule to the 3rd position of cyanidin; It is cyanidin-3-glucoside (He et al., 2010; Olivas-Aguirre et al., 2016). The anthocyanins in red grapes were usually identified as cyanidin-3-glucoside (Cy-3-glu or Cy3G), delphinidin-3-glucoside (Dp-3-glu or Dp3G), malvidin-3-glucoside (Mv-3-glu or Mv3G), pelargodin-3-glucoside (Pg-3-glu or Pg3G), peonidin-3-glucoside (Pn-3-glu or Pn3G) and petunidin-3-glucoside (Pt-3-glu or Pt3G) (Olivas-Aguirre et al., 2016; Türkmen Özen, 2015; Zhao et al., 2023). Anthocyanin amounts in some grape varieties and products were given in Table 2.

Table 2. Anthocyanin amounts in some grape varieties and products

Sources	**Varieties**	**Dominant Anthocyanin**	**Amount of Anthocyanin (mg L^{-1} or kg^{-1})**	**References**
Raisin	Antep Karası	Malvidin-3-glikozit	144, 4	Candemir et al., 2015
Skin	Cabernet Franc	Malvidin-3-glikozit	41,0	Costa et al., 2014
	Negro Mouro	Malvidin-3-glikozit	58,8	Costa et al., 2014
	Gamay	Malvidin-3-glikozit	17,7	Costa et al., 2014

Fruit	Öküzgözü	Malvidin-3-glikozit	563.11	Türkmen Özen, 2015
	Öküzgözü	Peonidin- 3-glukozit	40.84	Türkmen Özen, 2015
	Öküzgözü	Delfinidin3-glukozit	9.12	Türkmen Özen, 2015
	Öküzgözü	Siyanidin-3glukozit	16.30	Türkmen Özen, 2015
	Öküzgözü	Petunidin- 3-glukozit	11.04	Türkmen Özen, 2015
	Isabella	Malvidin-3-glikozit	438.17	Türkmen Özen, 2015
	Isabella	Peonidin- 3-glukozit	71.49	Türkmen Özen, 2015
	Isabella	Siyanidin-3glukozit	11.35	Türkmen Özen, 2015
	Cabernet Frank	Malvidin- 3-glukozit	94.1	Cliff et al., 2007
	Merlot	Malvidin- 3-glukozit	109.7	Cliff et al., 2007
	Pinot Noir	Malvidin- 3-glukozit	61.0	Cliff et al., 2007
	Cabernet Sauvignon	Malvidin- 3-glukozit	124.9-241.41	Cliff et al., 2007; Türkmen Özen, 2015
	Cabernet Sauvignon	Peonidin- 3-glukozit	15.27	Türkmen Özen, 2015
Seed	Baladi Black	Total monomeric anthocyanin	6,8	Rababah et al., 2008
Grape Juice	Red Globe	Total monomeric anthocyanin	525.91	Çağındı, 2016
	Cabernet Sauvignon	Total monomeric anthocyanin	299.34	Türkmen Özen, 2015
	Isabella	Total monomeric anthocyanin	37.24	Türkmen Özen, 2015
	Öküzgözü	Total monomeric anthocyanin	213.68	Türkmen Özen, 2015
	Syrah	Total monomeric anthocyanin	421.77	Türkmen Özen, 2015
	Papazkarası	Total monomeric anthocyanin	5.17	Türkmen Özen, 2015
Wine	Merlot	Total monomeric anthocyanin	305	Yüksel Küskü And Tahmaz Karaman, 2023
	Merlot	malvidin%	82.7	González-Neves et al., 2016
	Syrah	malvidin%	86.8	González-Neves et al., 2016
	Tannat	malvidin%	77.0	González-Neves et al., 2016
	Boğazkere	Toplam monomerik antosiyanin	212-409	Miran, 2018
	Öküzgözü	Toplam monomerik antosiyanin	243-437	Miran, 2018
	Kalecik Karası	Toplam monomerik antosiyanin	287	Seçen, 2021
	Cabernet Sauvignon	Toplam monomerik antosiyanin	311-357	Yue et al., 2021; Yüksel Küskü And Tahmaz Karaman, 2023
	Cabernet Frank	Toplam monomerik antosiyanin	315	Yüksel Küskü And Tahmaz Karaman, 2023

Grape variety, growing conditions and terroir are very effective in the amount of anthocyanins (García-Beneytez et al., 2002; Miran, 2018; Lago et al., 2023). Processing and storage conditions in grape products such as grape juice, wine, grape skin and seed extract, and the processing steps during their addition to foods also affect the structure of bioactive compounds (Türkmen Özen, 2015; Çağrı, 2016). On the other hand, anthocyanins in wine are mostly derived from grape berries; however, their stability and composition are affected by many factors such as pH, storage temperature, storage time, yeast, light exposure during the fermentation process etc. (Cliff et al., 2007; Yue et al., 2021; Seçen, 2021; Yüksel Küskü and Tahmaz Karaman, 2023).

4. CONCLUSION

In recent years, plants are important not only for their nutritional content but also for their phytochemical and phenolic contents. Especially, intensive research has been conducted on human health and longevity, and it appears that these ingredients are effective. However, some plants have gained importance in terms of secondary metabolites as well as high nutrients they contain. In this sense, anthocyanin is very important as a secondary metabolite and is abundant in grapes. Grape variety, environmental conditions, cultivation technique and method of obtaining the product are effective in the amount of anthocyanin. In this review, taking all these issues into consideration, anthocyanin in grapes and its importance were summarized.

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CHAPTER 4

THE PLANT OF IMMORTALITY "AMARANTH"

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Fig 1. Amaranthus caudatus

History of Amaranth Plant

It has been consumed as a staple food It has been consumed as a staple food through history, including the Inca, Mayan and Aztec civilisations (Caselato-Sousa ve Amaya-Farfán, 2012. Amaranth, which was widely cultivated and consumed in Latin America, was banned after the Spanish conquest and continued to be produced only on a small scale (Berghofer and Schoenlechner, 2002).

Taxonomy of Amaranth

Amaranth belongs to the genus Amaranthus and the family Amaranthaceae, which consists of 65 genera and 850 species. This genus consists of 50-60 species, the leaves of which are edible. The most important foliage vegetables in tropical countries in South Asia, South East Asia, east Africa, centre Africa, west Africa, Ethiopia, the Pacific and the Far East.

Important species of the amaranth plant are given below. These are:

Amaranthus tricolor L.

Fig.2.https://seedcorner.com/amaranthus-perfecta-amaranthus-tricolor-seeds/

Amaranthus dubius L.

Fig.3.https://tr.wikipedia.org/wiki/Amaranthus_dubius

Amaranthus lividus

Fig.4.https://botany.cz/cs/amaranthus-lividus/

Amaranthus blitum

Fig 5.https://getplanta.com/plant/flowering-plants / RXLochCmycoW4mDC2yya/ amaranthus-blitum

Amaranthus tristis L.

Fig 6.https://www.organicgarten.com/products/amaranthus-tristis/47710200000022884

Amaranthus spinosus L.

Fig7.https://mrec.ifas.ufl.edu/research/weedsbyflowercolorgreen/amatanthusspinosus/

Amaranthus viridis L.

Fig 8. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:316349-2

Amaranthus graecizans L.

Fig 9. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:10641-2

Amaranthus-hypochondriacus

Fig 10. https://indianmedicinalplants.info/medicinalplants-gallery/ index. php/Medicinal-Plants-of-America/Amaranthus-hypochondriacus

Amaranthus cruentus

Fig 11. https://pladias.cz/en/taxon/overview/Amaranthus%20cruentus

The most popular grain-like Amaranth species are *Amaranthus Hypochondriacus L., Amaranthus cruentus L.* and *Amaranthus caudatus L.*

Amaranthus hypochondriacus is a disease-resistant species that grows in temperate conditions. Due to its excellent nutritional composition, it can be roasted and ground like corn used in bread making at home (Mlakar et al., 2009). It is grown as an ornamental plant in Africa. It is used in the computer industry and cosmetics industry due to the oil contained in the starch granule (Schippers, 2002, Grubben and Denton 2004). It is also used in the pharmaceutical industry due to the active ingredient squalene found in its grain (He ve ark. 2002; He and Corke, 2003). It has been reported that squalene has beneficial effects on cancers (Rao and Newmark, 1998) and reduces the cholesterol level in the blood (Smith, 2000).

Amaranthus cruentus is a widely adapted plant with neutral photoperiod requirements. The protein of *A. cruentus* has amino acids including methionine and cysteine and high amounts of sulfur (Martinez-Nunez et al. 2019). This makes it a good combination with cereals that can be used for the formulation of complementary foods and snacks. Its leaves and tender stems are used in various dishes. As a way to add value to extend shelf life, the leaf can be dried and ground into a powder

Amaranthus caudatus, is grown at high altitudes in South America and Asia. Its seeds can be dried, ground and used in human nutrition in the form of whole grain amaranth flour, crackers, gluten-free brown bread, biscuits and cookies. Interspecific and intervarietal hybridisation in nature has resulted in a wide variation in the colour and shape of the leaves and flowers of the plant and even in the time between sowing and harvesting.

Botanical Characteristics of Amaranth Plant

Amaranth is a dicotyledonous plant belonging to the Amaranthaceae family. It does not belong to the same family as cereals, but it is classified as pseudocereal (cereal-like) because its nutritional composition and intended use are the same (Berghofer and Schoenlechner, 2002).

Amaranth is an annual herb, erect or creeping, with few or many branches. Some species have developed fringing roots and some have developed taproots. Stem color is green to purple or mixed shades of these two colors, glabrous and succulent; leaf simple, alternate or opposite, leaf tip lanceolate, leaf color purple in young leaves, green in mature leaves; inflorescence terminal and axil, colors can be different colors from green to dark purple and orange. Flowers small, regular, mostly unisexual, monoecious. Filaments free or united at the base, anthers one- or two-parted, style one, two or three-branched.

The proportion of male and female flowers varies during the flowering period. Each glomerulus consists of an initial staminate flower and an

indeterminate number of pistillate flowers. The extent and degree of cross-pollination is governed by the ratio of staminate and pistillate flowers within an inflorescence and the position of the inflorescence on the plant.

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Cultivated for its leaves, Amaranthus is said to be native to India; the centers of Amaranthus diversity are Central and South America, India and Southeast Asia, with secondary diversity in West Africa and East Africa. Among the leafy species, *Amaranthus tricolor* has a dominant position in India with different morphological forms in terms of color and leaf shape. Amaranthus cruentus is cultivated in Guatemala. The species *Amaranthus caudatus* is cultivated mainly in the Andes Mountains of the Americas and is also distributed in Asia and Africa. The nutrient content of amaranth leaves is given in the table below (Table 1).

Table 1. Nutrient content of Amaranth leaves (g/100 g).

Composition	**Quantity**	**Composition**	**Quantity**
Water (g.)	10.0	Leucine (mg/g N)	403
Crude Fiber (g.)	9.6	Histidine (mg/g N)	121
Protein (g.)	2.5-3.5	Valine (mg/g N)	241
Fat (g.)	0.31-0.5	Tryptophan (mg/g N)	59
Carbohydrate (g.)	4-6	Phenylalenine (mg/g N)	273
Iron (g.)	2.3-3.2	Tyrosine (mg/g N)	236
Calcium (mg.)	215-260	Methionine (mg/g N)	111
Vitamin C (mg/100 mg)	43-55	Potassium (mg/100 mg)	508

(Uusikua et al. 2010)

The chemical composition of Amaranth seed is shown in Table 2.

Table 2. Chemical composition of Amaranth plant seed

Bileşim	**Miktar**	**Bileşim**	**Miktar**
Energy (kcal/100 g)	371	Vitamin C (mg/100 g)	4-7
Moisture (%)	11.29	Iron (mg/100 g)	7.61
Protein (%)	13.56	Zinc (mg/100 g)	287
Oil (%)	7.2	Magnesium (mg/100 g)	248
Ash (%)	2.88	Manganese (mg/100 g)	3.3
Carbohydrate (%)	65.25	Potassium (mg/100 g)	508
Fiber (%)	6.7	Calcium (mg/100 g)	159

(Berghofer and Schoenlechner 2002; Caselato-Sousa, and Amaya-Farfán, 2012; Barba de la Rosa., et al. 2009; Valcárcel Yamani B., et al.)

As can be seen from the tables, Amaranth leaves are higher in Vitamin C, fiber and Calcium than seeds, but lower in protein, fat and carbohydrates.

Sowing

Amaranthus is grown from seed. To grow plants outdoors, planting should be done after the last frost dates of the region, when the soil begins to warm up. The sowing time for our country is mid-March for the western and southern regions and mid-April for the eastern and northern regions. If Amaranthus is to be grown for seed, 200 g/ha of seed should be used; if it is to be grown for grass, 100 g/ha of seed should be used. 40-50 plants per square meter is ideal.

Since the seeds of the Amaranthus plant are small, the soil should be thoroughly broken up and crumbled in order to ensure easier germination. Good emergence could not be achieved when sowing in cloddy soils. The application of burnt barnyard manure before plowing the soil gives very good results for seed and seedling development. Amaranth sowing can be done with a grain seeder, hand sowing in rows or sprinkling sowing and seedling planting.

If direct sowing is to be done, two methods are applied. These are row sowing and sprinkle sowing. In row sowing, rows are opened at 50 cm intervals and seeds are sown in these rows by hand. A gap of at least 20-25 cm should be left between the two seeds. Then the seeds should be covered with about 1-1.5 cm of soil. If the sowing is to be done as sprinkling, the seeds are mixed with 10 parts of sand for even distribution of the seed and scattered on the field. Afterwards, the soil is not cultivated with any tool. If planting is to be done as a seedling, planting is done by leaving 50 cm between rows and 20-25 cm above the row, as in seed planting. Care should be taken to ensure that the seedlings are 15-20 days old.

Climate and Soil requirements

Amaranth can grow in a wide range of climatic conditions (Chauhan ve ark., 2016). However, it is sensitive to temperatures below 0°C. A temperature range of 20-30°C is ideal for good vegetative growth. A. caudatus, A. cruentus and A. edulis are short-day species, while the commonly cultivated cereal Amaranthus species, namely A. hypochondriacus, are neutral-day plants. However, all species have early and late varieties. Grain-type Amaranthus are highly drought tolerant. Leafy Amaranthus is best grown in the plains.

The most suitable soils for growing Amaranthus are loamy soils with good drainage, close to neutral pH (Sagar et al., 2023). However, they can be grown in all soils except heavy clay soils.

Fertilizer and Water Requirement

Amaranth responds very positively to fertilizers and organic matter. For amaranths to be grown for seed, it is recommended to add 2.5 tons of burnt farm manure per decare and mix it with the soil before tilling the soil, and then apply pure fertilizer at 5 kg N/da, 5 kg P/da and 5 kg K/da. For the Amaranth plant to be grown for its leaves, 2.5 tons of burnt farm manure per decare should be mixed into the soil before planting, then 3 kg N/da, 5 kg P/da and 5 kg K/da fertilizer should be applied with planting, and 3 kg N/da eight weeks after planting. It is also recommended to give . It has also been suggested that for high seed yield, leaf harvest should be done once 30 days after planting (Yawalkar and Ram 2004). Since the frequency of irrigation will increase during hot and dry summers, the amount of nitrogen in the soil must also be increased. In such cases, up to 12 kg N/da of fertilizer can be applied to the soil.

The crop should generally be planted in a field with sufficient moisture. However, if the moisture in the field is not sufficient during planting, the first irrigation should be done immediately after planting and then again on the third day. Subsequent irrigations are carried out at intervals of 3 to 5 days in summer and as needed in rainy seasons.

Drip irrigation is recommended as an irrigation method. Weed growth was also found to be less in lands irrigated with drip irrigation (Aras, 2006). As irrigation frequency and nitrogen levels increased, protein content also increased.

Treatments

Regular pulling of weeds in the early stages of cultivation reduces competition for nutrients, light, moisture and space between the plant and weeds. Thus, the plant can grow better. In addition, hoeing in the early stages not only destroys weeds, but also ensures better aeration of the soil. This

should be done until the plant reaches a height of 25-30 cm from sowing. After 10-15 days after sowing, thinning should be done so that 20 cm space is left between the plants. At the seedling stage, one fungicide spraying will be useful to prevent the plant from getting fungal diseases. At the seedling stage, one fungicide spraying will be useful to prevent the plant from getting fungal diseases.

Harvest

If harvested as leaves, the plants must be collected with their roots on the 25th day from the planting date, and if they are to be harvested as stems, the plants must be collected with their roots on the 40th day from the planting date. In amaranth, the grains ripen early, but the whole plant dries quite late. If the ears are left until the plant dries, it will cause excessive grain fragmentation and heavy grain loss. This is why it is important to minimize grain loss when the seeds begin to dry. Therefore, in order to minimize grain loss when the seeds start to dry, the head of the plant is cut off in the morning when the sun rises and the plant is a little wet, these heads are pounded to extract the seeds, and these seeds are left in the sun for 6-7 days to dry thoroughly. The product will be ready for harvest within 80-120 days, depending on the variety and season. A healthy crop with good management practices provides a grass yield of approximately 20 to 40 tonnes per hectare, depending on the variety. The crop planted in March yields higher yields than the crop planted in June.

Uses:

Amaranth is mostly used for seed production in the USA, but it is also used for different purposes in other parts of the world. In Africa and the Caribbean, the leaves of Amaranth are harvested at certain times and consumed as greens. In China, farmers use the plant as fodder for large pigs and cultivate over 100,000 acres. The vast majority of Amaranth produced in the US is used as mill flour, mixed with wheat flour or other flours to make breakfast cereals, crackers, cookies, bread and other bakery products. Utilization rate studies have shown that Amaranth can be blended with other flours to 50% or even 75% in bakery products without any change in properties or taste. FAO statistics do not list the production amount.

Amaranth contains about 13-14% protein. The protein content of amaranth is usually higher than that of cereals.Amaranth proteins consist of 40% albumin, 20% globulin, 2-3% prolamin and 25-30% glutenin.Amaranth globulins have been reported to have some functional properties such as emulsifying properties (Berghofer ve Schoenlechner, 2002). The lysine amino acid content of amaranth is rich compared to cereals such as corn, rice and wheat. In addition, the amount of sulfur amino acids in amaranth (2-5%) is higher than the amount of sulfur amino acids normally found in pulses at

1.4%. Amaranth, which contains amino acids such as alline, valine, leucine, arginine, arginine, phenylalanine, pralines, methionines, α aminobutyric acid, tryptophan, isoleucine and serine, is a quality protein source (Rastogi and Shukla, 2013). The protein content of amaranth is very close to the level recommended by FAO/WHO for a balanced diet (Maurya and Arya, 2018). The fact that it contains the amino acids histidine and arginine, which are essential for infants, makes amaranth important in infant nutrition (Berghofer and Schoenlechner, 2002).

Amaranth contains approximately 7% fat. The amount of fat contained in amaranth is higher than the amount of fat contained in grains. The ratio of saturated fatty acids to unsaturated fatty acids in amaranth is 1:4 (Maurya and Arya, 2018). Linoleic acid constitutes most of the unsaturated fat content of amaranth, which has a high rate of unsaturated fat. 50% of the total oil is linoleic acid, 20% oleic acid, 20% palmitic acid and approximately 1% linolenic acid. Phospholipids constitute 5% of the fat (Berghofer and Schoenlechner, 2002; Rastogi and Shukla, 2013).

Amaranth contains about two times more minerals than cereals. Amaranth also contains more minerals than buckwheat. 66% of these minerals are found in the bran and embryo layers. It contains especially high amounts of calcium, phosphorus, iron, magnesium, potassium, sodium and zinc (Mlakar and ark., 2009). Phosphorus in amaranth is produced in the presence of phytic acid. The amount of phytic acid in amaranth varies between 0.3-0.6%. Phytic acid has also been shown to lower cholesterol levels in humans (Rastogi and Shukla, 2013). Amaranth contains significant levels of vitamins and is a good source of daily essential vitamins. It contains more riboflavin and vitamin C than cereals. It is also a good source of vitamin E, which has antioxidant properties (Berghofer and Schoenlechner, 2002). Recent studies have focused on the bioactive components contained in amaranth because of their potential to reduce the risk of chronic diseases. Amaranth contains bioactive phytochemicals such as phenolic compounds, betanins and carotenoids.

As the world takes steps to tackle hunger, poverty and food insecurity, amaranth grain is being recognized again, especially for the African continent. However, its full potential has yet to be exploited. Continuous and detailed efforts in research and development are needed, as well as the coordination of all stakeholders for the effective implementation of relevant actions (Devaux et al., 2018).

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CHAPTER 5

USE OF SOME INDEXES FOR THE WATER QUALITY ASSESSMENT OF SURFACE WATERS IN TURKEY: AN OVERVIEW

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Introduction

In today's rapidly deteriorating environmental conditions, changes are observed in the physical, chemical, and biological characteristics of water. As a result of these changes, monitoring of water quality is necessary for the evaluation of its physical, chemical, and biological features, as well as for the detection of current conditions and potential trends (Pulatsü et al. 2014). Various anthropogenic activities such as industrialization, urbanization, sewage, industrial wastewater, and agricultural activities encourage the entry of heavy metals into water sources. Monitoring is required not only for physical, chemical, and biological components but also for toxicological elements such as heavy metals, considering the ecosystem and human health (Pulatsü and Topçu 2015; Real et al. 2024). Health risks, both non-carcinogenic and carcinogenic, arising from potential exposure to heavy metals pose a negative threat to human health. Due to its importance, a number of indices developed for health risk assessment (hazard quotient (HQ), hazard index (HI) and cancer risk index (CR)) have been addressed in studies on the quality of surface waters in Turkey in recent years (Baştürk 2019; Varol 2019; Tokatlı and Ustaoğlu 2020; Ustaoğlu et al. 2020a; Varol et al. 2021; Pulatsü and Latifi 2023b; Tokatlı et al. 2023; Varol and Tokatlı 2023).

However, since many parameters affect water quality, the simultaneous interpretation of parameters makes the process of determining water quality a costly, complex and time-consuming process. For this reason, water quality indices (WQI) are used for simpler and more understandable interpretation of water quality, and it is possible to determine or change environmental policies based on the index findings (Tirkey et al. 2013; Ameen 2019). The main purpose of the WQI is to convert data on constituents and concentrations in a sample into a number so that a single value for water quality can be established. Thus, by looking at the index value of each sample, it is possible to recognize changes in water or make comparisons between different water bodies (Nowicki 2020). Generally, water quality indices are analyzed under five main groups. The first is the general water quality indices, which do not take into account water consumption and are used to assess

overall water quality. The second is specific water quality indices, where water quality is determined for specific uses (drinking, irrigation, industrial, etc.). Third, there are design or planning indices used in water quality management and decision-making. Fourthly, statistical indices are created by minimizing personal opinion and using the findings of statistical methods. The fifth is biological indices, which are used to determine the effects of water quality on aquatic life (Asadollahfardi 2015). Many organizations have created their own water quality indices using different parameters for the determination and control of water quality. Table 1 presents the commonly used methods for water quality indices applied in the studies covered in this study. However, the parameters taken into account in different studies may vary according to the studies, and the indices used may also be modified.

This review focuses on national studies on different water quality indices that have been widely used in recent years to determine the quality of surface waters in Turkey and the current status of their application. For this purpose, different water quality indices, heavy metal indices, health risk assessment indices applied in different surface water sources (rivers, lakes, dam lakes, streams) are presented in Table 2, and the parameters selected in the calculation of the indices and the main results of the studies are summarized.

Table 1. Parameters used in the calculation of different water quality indices and quality classification according to indices

Index	Formula	Water Quality Class	Reference
Weighted Arithmetic Water Quality Index (WA-WQI)	$WA\text{-}WQI = \frac{\sum QiWi}{\sum Wi}$	0–25:Excellent water quality; 26–50:Good water quality; 51–75:Poor water quality; 76–100:Very poor water quality; Above 100: Unsuitable for drinking purpose	Tyagi et al. 2013

Table 1 (continued)

Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI)	CCME-WQI = $100 - \left(\frac{\sqrt{F_1^2+F_2^2+F_3^2}}{1.732}\right)$	95-100: Excellent; 80-94.9: Good; 65-79.9: Fair; 45-64.9: Marginal; 0-44.9: Poor	CCME (2001)
Oregon Water Quality Index (OWQI)	OWQI = $\sqrt{\frac{n}{\sum_{i=1}^{n}\frac{1}{SI_i^2}}}$	90-100: Excellent; 85-89: Good; 80-84: Fair 60-79: Poor; 10-59: Very Poor	Cude (2001)
National Sanitation Foundation-Water Quality Index (NSF-WQI)	NSF-WQI = $\sum_{i=1}^{n} QiWi$	91-100: Excellent; 71-90: Good; 51-70: Medium; 26-50: Bad; 0-25: Very bad	Brown et al. (1970)
Dinius Water Quality Indes (D-WQI)	$DI=\frac{1}{21}\sum_{i=1} W_i I_i$	0-49%: Not Acceptable 50-59%: Doubtful 60-80%: Necessary treatment becoming more extensive 81-90%: Minor Purification required 91-100%: Purification not necessary	Dinius (1972)
Organic Pollution Index (OPI)	OPI= $\frac{COD}{CODs} + \frac{DIN}{DINs} + \frac{DIP}{DIPs} - \frac{DO}{DOs}$	<0: Excellent; 0-1: Good; 1-2: Begin to be contaminated; 2-3: Lightly polluted; 3-4: Moderately polluted; 4-5: Heavily polluted	Quan et al. (2005)

Table 1 (continued)

Aquatic Toxicity Index (ATI)	$ATI = \frac{1}{100}(\frac{1}{n}\sum_{i=1}^{n} qi)^2$	0-50: Totally unsuitable for normal fish life 51- 59: Suitable only for hardly fish species 60- 100: Suitable for all fish life	Wepener et al. (1992)
Nutrient Pollution Index (NPI)	$NPI = (C_N/MAC_N) + (C_P/MAC_P)$	< 1: No pollution; 1 – 3: Moderate polluted 3 – 6: Considerable polluted; > 6: Very high polluted	Isiuku and Enyoh (2020)
Heavy Metal Pollution Index (HPI)	$HPI = \frac{\sum_{i=1}^{n} WixQi}{\sum_{i=1}^{n} Wi}$	Critical pollution index value is 100. Less than 100 is considered suitable for drinking.	Mohan et al. (1996)
Heavy Metal Evaluation Index (HEI)	$HEI = \sum_{i=1}^{n} \frac{Hc}{H_{MAC}}$	HEI < 10: Low HEI = 10–20 : Medium HEI > 20 High	Edet and Offiong (2002)

Table 2. Index application studies on different surface water resources in Turkey

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
WQI	Tahtalı Reservoir (İzmir)	Cd, CN^-, Hg, Se, As, F^-, NO_3-N, DO, BOD, TP, pH, Total coliform	- The findings showed that the overall quality of surface water was classified as 'excellent'. - Water quality was strongly influenced by agricultural and domestic uses. - This technique is reported to help decision makers to report the status of water quality and also to investigate spatial and temporal changes.	Boyacıoğlu (2007)
WQI	Karaçomak Dam (Kastamonu)	EC, pH, WT, DO, Turbidity, TH, TA, NH_4 -N, NO_2 -N, NO_3 -N, PO_4 -P, BOD, COD	-The WOI values for two selected stations, namely the drinking water supply entry point in Kastamonu city (S1) and another entry point along the main dam for approximately (449) meters (S2), were found to be 35.5 and 32.4, respectively. -The importance of using microbiological parameters together with physico-chemical parameters in WQI calculations and the main priority of water quality monitoring were emphasized.	Imnesisi and Aydın (2016)

Table 2 (continued)

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
HPI	Çamlıdere Dam (Ankara)	Pb, Zn, Cr, Mn, Fe, Cu, Cd, Co, Ni, Al, Hg, As, Mo, Sb, Se, B, Be, Ag, Ba, Tl	-It was found that the heavy metal index values for three different branches feeding Çamlıdere Dam, one of the largest drinking water sources of Ankara, corresponded to the moderate class and the surface waters were not completely contaminated with trace elements. -It has been reported that the use of heavy metal pollution index, which includes many trace elements, is useful in evaluating the pollution level according to heavy metals in Çamlıdere area.	Tunç Dede (2016)
D-WQI HRA***	Mamasın Dam (Aksaray)	BOD, NO_3, pH, DO, WT, EC, Cl, fecal coliform, total coliform, TA, TH, Cl, color	-The water quality of Mamasın Dam, the drinking water source of Aksaray, was found to be low (<60) according to WQI values. It has been reported that Mamasın Dam water quality is only suitable for agricultural (irrigation) and industrial use and cannot be used for drinking water supply without treatment. - HRA: The non-carcinogenic health risks of As (Arsenic) pose a 'moderate' and 'high' level threat to both adults and children through ingestion exposure pathway. The elements B, F, and Pb have Hazard Index values above 0.1, and it has been stated that when taken through ingestion, they may cause a 'low' level risk of chronic illness in children.	Baştürk (2019)

Table 2 (continued)

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
WQI	Saraydüzü Dam Lake (Sinop)	Cd, Cl, Cu, Pb, Hg, Ni, NO_3, NO_2, Na, TH, pH, SO^4, Zn	- WQI values were determined between 17.62 and 29.88. - According to the results of factor analysis (FA), pH, temperature, EC, SSM, BOD, TH, TA, Ca+2, NO3, NH4, Hg and DO were reported to be the main variables responsible for the processes in the ecosystem.	Kükrer and Mutlu (2019)
HPI	Alakır Dam-Alakır Bridge (Antalya)	As, Mn, Ni, Cu, Pb, Fe, Sr, Cr	- It was stated that the increases in the HPI values in the samples taken from 48 locations between Alakır Dam and Alakır Bridge were concentrated in two regions and that the dam in the upper region and agricultural activities in the lower region were effective in this grouping.	Leventeli and Yalçın (2019)

Table 2 (continued)

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
HRA	Keban Dam Lake (Elazığ)	Co, Cr, Cu, Fe, Mn, Ni, Zn, As, Cd, Pb	- The non-carcinogenic and carcinogenic risks through ingestion and dermal contact exposure pathways were estimated for dissolved trace metals in the reservoir water, which is of international importance due to its location on the Euphrates River, an important transboundary river. Hazard index (HI) and total HI values were less than 1 and carcinogenic risk (CR) values and total CR values of As and Cr were found to be within the acceptable range for both routes of exposure. - The researcher concluded that Keban Dam Lake is safe for human health considering residential and recreational uses.	Varol (2019)
HRA	Keban Dam Lake (Elazığ)	Pb, Hg, Cd, As, Cr, Ni, Co, Mn, Cu, Fe, Al, Sr, U, V, Zn, Zr, Ba	- All HQ (hazard quotient) and HI (hazard index) values for the deep waters of Keban Reservoir, the second largest reservoir in Turkey, were below the risk threshold. HI values for children were higher than those for adults. - The findings showed that HMs in the surface and deep waters of the reservoir do not pose a health risk for domestic and recreational use.	Canpolat et al. (2020)

Table 2 (continued)

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
WAI-WQI	Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy ve Süloğlu Dam Lakes (Thrace Region)	Li, Be, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, Tl, Pb	-According to the WAI-WQI results, it was determined that the reservoirs had 'Class A' water quality, and the values were within the permissible limits (<100). -The Qi values for selenium were found to be above the permissible limits, and the overall ranking of elements in the reservoir water was generally determined as Se > As > Cr > B > Mo > Ba > Ni > Pb > Cd > Mn > Cu.	Tokatlı (2020)
WQI	Yassıalan Dam Lake (Karadeniz Region)	pH, EC, Cl, NO_2, NO_3, SO_4, Na, Pb, Cu, Cd, Hg, Ni, Zn	-The reservoir was found as “A Grade – Excellent”; 1st – 2nd Class water quality in general.	Mutlu et al. (2021)

Table 2 (continued)

Index*	Dam Lakes-Reservoirs	Parameters**	Major Findings	Reference
WQI OPI	Kışla (Kozcagiz) Dam (Bartın)	pH, EC, COD, BOD, Cl^{-}, PO_4^3, SO_4^{-2}, Na^{+}, K^{+}, TH, HCO_3^{-}, Mg^{+2}, Ca^{+2}, NO_2–N, NO_3-N	The WQI results of the reservoir located in the Western Black Sea Region show that the water quality is generally good in all seasons; only sampling points S1 (101.58), S2 (100.59), S4 (102.31) and S5 (102.12) showed poor water characteristics in the fall. - According to OPI results, good water quality was obtained in winter and spring, while summer samples were found to be slightly and fall samples moderately contaminated.	Şimşek and Mutlu (2023)

Index*	Lakes	Parameters	Major Findings	Reference
HPI	Van Lake Basin	Al, As, Co, Cr^{+6}, Fe^{+2}, Mn^{+2}, Mo, Ni, Zn	-According to the HPI (Heterotrophic Plate Index) quality classes for the 7 brackish water sources in the lake basin, no sampling point was found in the 'excellent' class, and only one sampling point was determined to be in the 'good' class. The rest of the sampling points were classified as 'unusable water. - When the findings obtained were evaluated with water quality regulations, it was found that it was not appropriate to use water from these sources for agriculture and breeding purposes.	Atıcı et al. (2021)

Table 2 (continued)

Index*	Lakes	Parameters	Major Findings	Reference
CCME-WQI	Aygır Lake (Bitlis)	WT, DO, EC, pH, NH_3, F, As, TA, TH	- CCME-CWQI value was calculated as 83.30±5.74 and classified as good quality waters. -Due to the low population around the lake and the absence of industrial facilities in the lake vicinity, it is stated that the index could be of 'good' quality.	Çavuş and Şen (2020; 2022)
$WQI_{fisheries}$		WT, DO, EC, pH, NH_3, TA, TH, turbidity	- The AG-WQI drinking value for Aygir Lake was 149.41 and the AG-WQI fisheries value was 76.11, indicating that the lake water is suitable for drinking and fishing.	
HPI	Beyşehir Lake (Konya)	As, Cr, Cu, Ni, Zn, Pb, Cd, Hg, Fe, Al, Mn	- It is stated that the lake waters, which is the largest freshwater lake and drinking water reserve in Turkey, are below the limit values in terms of heavy metals and are suitable for drinking water according to the HPI value.	Şener et al. (2023)
HEI			- According to HEI and Cd values, all of the samples were classified as "low pollution".	

Table 2 (continued)

Index	Lakes	Parameters	Major Findings	Reference
HPI HRA	Ponds (Saros Bay)	Cr, Mn, Ni, Cu, Zn, As, Cd, Pb, B	- Water Quality Index (WQI) results of dry (late summer) and wet (late winter) surface water samples collected from 9 stations from 5 basins, namely Anadere, Çorlu, Tunca, Meriç and Ergene Rivers, revealed that the most polluted components of the basin are Çorlu Stream and Ergene River. - Organic pollution risk assessment indices as follows: Çorlu Stream>Ergene River>Anadere Stream>Tunca River>Meriç River. - In order to improve the quality and sustainability of aquatic life in the Meriç-Ergene River Basin, it is recommended to avoid the unconscious use of pesticides and fertilizers in the region and to prevent the discharge of industrial wastewater and municipal sewage without treatment.	Tokatlı et al. (2023)

Index	Rivers - Streams	Parameters	Major Findings	Reference
CCME-WQI	Kucuk Menderes Basin (İzmir)	pH, TDS, Cl, NO_3–N, DO, BOD_5, SO_4, B	- Surface waters in the basin are mostly categorized as water class A2. - The modified categorization helps water managers to integrate and interpret the overall picture of the CCME-WQI.	Boyacıoğlu (2010)

Table 2 (continued)

Index	Rivers - Streams	Parameters	Major Findings	Reference
CCME – WQI OWQI NSF – WQI	Streams (Sapanca Lake Basin)	WT, pH, EC, DO, TDS, TSS, Ca, Mg, Cl, SO_4, O-PO_4-P, NO_3-N, NO_2-N, BOD_5, COD	- The most important and largest streams that drain into the Sapanca Lake are the Balikhane, Karacay, Kurucay, Mahmudiye, Istanbul, Keci, Sarp, and Arifiye Streams and the Cark (Beskopru). The pollution in the streams feeding into Sapanca Lake is reported to be more effectively determined by modified WQI scores when compared with CCME-WQI, OWQI, and NSF-WQI. - Within the scope of the study, an alternative index called WQIeut (eutrophication) was developed using DO, O-PO4-P, NO3-N, NO2-N, BOD5 and COD parameters. A good correlation was found between the developed indices and the fifteen-parameter WQI. - The research revealed the danger of eutrophication for Sapanca Lake and the streams that feed it.	Akkoyunlu and Akiner (2012)
CCME-WQI OWQI ATI	Kirmir Basin (Ankara)	pH, DO, turbidity, NH_4, total dissolved salts, Fl, K, o-PO_4, total Zn, Mn, Cr, Cu, Pb, Ni	-5 different water quality indices were determined in water samples taken from 10 sampling stations in the basin for one year and it was concluded that CCME-WQI and O-WQI provided the best results.	Tunç Dede et al. (2013)

Table 2 (continued)

Index	Rivers - Streams	Parameters	Major Findings	Reference
HPI	Bogacayi River (Antalya)	As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Sr, V	-The HPI values were found to be in the range of 7.81 to 43.97, with an average of 25.48. The fact that all HPI values are below 100 indicates a critical HPI value for drinking safety.	Cengiz et al. (2017)
WQI	Aksu River (Antalya-Isparta)	pH, HCO_3, Cl, SO_4, Na, Ca, Mg, COD, NO_3, NO_2, Pb, Cr, Mn	- Due to the planning of obtaining drinking water from Karacaören-1 Dam Lake in Antalya Province, the WQI values calculated for the drinking water quality of Aksu River, which is the main river feeding Karacaören-1 Dam Lake and located approximately 145 km from Isparta Province to the Mediterranean Sea, ranged between 35.6133 and 337.5198. -While it was stated that the Karacaören-1 Dam Lake generally has good water quality, it was reported that the water quality is poor and very poor in the north and south of the river basin.	Şener et al. (2017)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
CCME-WQI	Coruh River Basin (Eastern Black Sea Region)	pH, EC, TDS, Na, K, TH, Mg, HCO_3, TA, Cl, SO_4, NH_4-N, NO_2- N, NO_3-N, PO_4, DO, BOD, MnO_4, turbidity, TP, SS, color	- Water quality data measurement results obtained by the 26th Regional Directorate of State Hydraulic Works from four different regions in the Çoruh River Basin for four years between 2011 and 2014 were used. CCME-WQI value was calculated as 30.4 and 71.35 and the quality of the waters was classified as "poor", "marginal" and "fair". The findings revealed that surface waters in the Çoruh River Basin were degraded or under threat, and their overall quality was not close to natural or desired levels	Bilgin (2018)
WQI	Mert Stream (Samsun)	WT, pH, EC, DO, TSS	- The average calculated WQI value was 81.9 (water classification of medium-quality), indicating a not too bad quality for the river water. The WQI values for Ist1, Ist2, Ist3, Ist4, Ist5, and Ist6 were found to be 91.6, 92.5, 74.3, 91.6, 75.2, and 62.5, respectively. The 5th and 6th stations located in the urban section of the river, along with the 3rd station in the rural area of the river, are under pollution pressure. At the 5th and 6th stations, the reason for the low water quality in the river water was domestic and industrial wastes, while the reason for the low quality at the 3rd station was stated to be the wastes poured into the river from the chicken farms located near the station in Kavak district.	Maraşlıoğlu et al. (2018)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI	Çağlayan, Fırtına and İkizdere Rivers (Rize)	WT, EC, DO, pH, $CaCO_3$, permanganate index, NO_2–N, NO_3-N, NH_4 –N, o-PO_4, TP, TSS, Chl-a, LAS, As, Cr, Cu, Mn, Ni, Pb, Zn	-The rivers are reported to be classified as "excellent" water quality according to WQI values and have a range of values suitable for drinking water. -It has been reported that the hydroelectric power plant on the Ikizdere River may have a significant impact on dissolved and particulate material distribution.	Alkan et al. (2019)
NSF – WQI CCME – WQ O-WQI	Karasu River (Aksaray-Central Anatolia)	DO, Fecal coliform, pH, BOD_5, NO_3, PO_4, T (°C), TDS, turbidity	- The general water quality of the river, which is one of the important drinking water sources of Aksaray Province, was evaluated with three different water quality index models. The water quality of Karasu River was found to be "moderate" according to NSF and "poor" according to CCME-WQI and O-WQI.	Alver and Baştürk (2019)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
NSF – WQI	Melendiz River (Aksaray)	DO, Fecal coliform, BOD_5, NO_3, PO_4, pH, turbidity, TDS, T (°C)	- When the physico-chemical and microbiological parameters were evaluated according to NSF and D-WQI, it was determined that the condition of the water source was "moderate (63.834)" and "poor (55.223)".	Baştürk and Alver (2019)
D-WQI			-According to the WQIs, it is concluded that the water source can be used for irrigation and industrial purposes in its current state and as drinking water after treatment.	
WQI	Zerveli Stream (Kastamonu)	28 different water quality parameters	-WQI values for monthly surface water samples from eleven stations ranged from 17.26 (excellent) to 223.05 (very poor). -It has been reported that water quality decreases with distance from the source, and the reason for this is that there are more settlements close to the sea and anthropogenic inputs are higher.	Mutlu (2019)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI	Kızılırmak River (Sivas)	BOD, Ca, Cl, DO, Fe, K, HCO_3, Mg, Mn, Na, NH_4, NO_2, NO_3, pH, SO_4, TDS, TH, TP	- The WQI spatial distribution maps calculated for wet and dry seasons and the final surface water quality maps clearly showed that the surface water quality of the Kızılırmak River is suitable for drinking purposes. The water quality at the extremities of the tributaries of the Kızılırmak River is in the "excellent water" category. The southern part of the Kızılırmak River, located south of the city center of Sivas, was in the "low water" category during the rainy season and in the "very poor water" category during the dry season. In particular, it was pointed out that agricultural activities and urban wastewater discharges would have a negative impact on the water quality of the Kızılırmak River.	Karakuş (2020)
WQI HPI HEI C_{deg} HRA	Meriç River Delta Wetland (Thrace Region)	Cr, As, Cd, Pb, Ni, Cu, Zn	- According to WQI, HPI, HEI and C_{deg} results, Ni and As were found to be the most hazardous toxic substances and Lake Gala was recorded as the most affected aquatic environment. - According to HQs and CR values, As was found to be the most dangerous toxic substance. -The most affected components of the Meriç River Delta are reported to be Gala Lake and the Ergene River.	Tokatlı and Ustaoğlu (2020)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI HRA	Turnasuyu Stream (Eastern Black Sea Basin)	NO_3-N, NO_2-N, NH_4-N, Cl^-, TH, TDS, EC, pH, SO_4, Cu, Al, Fe, Mn, Zn	- It was stated that the surface waters at three sampling points selected to represent the upstream, middle and downstream basins of Turnasuyu Stream have very good water quality characteristics based on WQI values. The HQ and HI values also indicate that trace elements are not in dangerous limits for public health.	Ustaoğlu et al. (2020a)
WQI	Çömlekçi Stream (Giresun)	pH, TDS, TH, Cl, NO_2-N, NO_3-N, SO_4^{-2}	-The WQI values ranged between 33.52 and 43.3. When considering all months and stations, the stream water quality has been classified as "good." -It indicates that the water quality is suitable for use in drinking water, agriculture, livestock activities, and aquatic life. Continuous monitoring of stream water quality parameters is recommended.	Ustaoğlu et al. (2020b)
WQI	Sürgü Stream (Euphrates River Basin)	WT, EC, DO, pH, TN, TP, TSS, COD, NO_2–N, NO_3-N, NH_4 –N, Cl, Ca^{+2}, Mg^{+2} SRP, SO_4^{-2}	-The WQI values for five selected points along the stream, used for drinking water source, agricultural irrigation, and rainbow trout production, ranged between 87.6 and 95.3, indicating "good" and "excellent" water quality.	Varol (2020)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI	Büyük Menderes River	pH, EC, TDS, Cl^{-1}, NO_2 –N, NO_3-N, NH_3 –N, DO, COD o-PO_4, SO_4^{-2}, Na^{+1}, K^{+1}, Ca^{+2}, Mg^{+2}	-While the WQI values ranged from 37.27 to 85.96, the average values calculated by month ranged between 56.88 and 71.38. According to the WQI values, the status of river water quality varies between "good" and "very poor." - While the water quality status was classified as "good" at the stations located upstream and downstream of the river route, it was classified as "poor" at the stations located in the middle section of the river. This is mostly because urban and rural settlements and all commercial activities are clustered in the central part of the river route. -To prevent river pollution and preserve water quality, it has been recommended to treat and purify wastewater from domestic sources and industrial activities before discharge, treat fertilizers before release, and regulate the use of pesticides in agriculture to reduce their exposure to water throughout the watershed.	Yılmaz et al. (2020)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI	Pazarsuyu, Batlama, Aksu, Yağlıdere, Gelevera, Harşit, Görele Streams (Giresun)	pH, EC, BOD_5, Cl^{-1}, SO_4^{-2}, TH, NO_2–N, NO_3-N, TDS, Na^{+1}, K^{+1}, Ca^{+2}, Mg^{+2}	-The annual average WQI values for the seven major rivers in Giresun Province ranged from 25.69 (excellent) to 32.39 (good). Based on these indices, it is concluded that settlements and related anthropogenic activities along the river banks have not yet negatively affected water quality.	Aydın et al. (2021)
WQI	Hasanağa Stream Basin (Edirne)	DO, Oxygen saturation, pH, EC, TDS, salinity, turbidity, PO_4, NO_3, NO_2, SO_4	- According to the WQI results for seven stations selected in the basin, although it was determined that the water quality decreased significantly in 2020, the basin water quality was generally reported to have "Class A - Excellent" water quality (<50). - It has been stated that for the protection and sustainability of the basin, local people must be constantly monitored and awareness-raising in agricultural activities.	Tokatlı (2021)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI HPI HEI	Emet River Basin	Ni, Cr, As, Zn, Pb, Cu, Cd	- According to WQI, HPI, HEI and Cdeg results, As and Cr were recorded as the most hazardous toxic substances in the samples taken seasonally from 8 locations. The seasonal mean values of the ecological indices applied to the watershed water ranged between 71.41-888.29 for WQI, 85.97-915.76 for HPI, 4.04-49.80 for HEI and - 2.95-42.80 for Cdeg. In addition to the intensive agricultural practices in the region, the geological structure of the basin was also evaluated as the main cause of high heavy metal accumulations in the basin waters.	Tokatlı et al. (2021a)
WQI HEI	Şehriban Stream (Black Sea Region)	Fe, Pb, Cu, Cd, Hg, Ni, Zn	- According to the WQI and HEI values, it was determined that the stream showed "Class A - Excellent <50) and Low Pollution" (<10), although the water quality decreased slightly in the fall. - It was suggested that unconscious use of fertilizers and pesticides should be avoided in the region, environmental seminars should be organized in coordination with local administrations to raise the environmental awareness of local people, and the water quality of Şehriban Creek should be continuously monitored in short periods.	Tokatlı et al. (2021b)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI HPI HI NPI	Terme River (Black Sea Region)	Na, Mg, K, Ca, Al, Cr, Fe, Co, Mn, Ni, Zn, Cd, Cu, Pb, As	-The determined values are WQI: 22, HPI: 15.61, HEI: 0.78, and NPI: 0.404, indicating that the overall water quality of the Terme River is classified as "good." - HQ and HI values were calculated below the risk threshold (<1). HI-total values were higher in children (2.48E-01) than in adults (2.14E-01), indicating that children's health is at higher risk than adults.	Ustaoğlu et al. (2021)
HRA	Karasu River (Eastern Anatolia)	Fe, Al, Cd, Ni, Cu, Zn, Pb, As, Mn, Cr	-In the monthly water samples taken from 8 stations along the main branch of the Euphrates River, the longest river in Southwest Asia, 10 trace elements were found to have HQ values below the risk threshold for both water intake and dermal contact pathways for the local residents. -The HI values, on the other hand, indicated that the ingestion of trace elements could pose a non-carcinogenic health risk for children. -The carcinogenic risk results for both water intake and dermal absorption of As and Cr were found to be within or below the acceptable carcinogenic risk range.	Varol et al. (2021)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WAI-WQI CCME-WQI UWQI OWQI ATI	Büyük Menderes River (Aydın Söke Basin)	Al, NH_4 -N, As, Ba, BOD, B, Cd, Ca, COD, Cl, Cr, Co, Cu, Pt-Co, DO, E-Coli, EC, F-Strp, Fe, Pb, Mg, Mn, Hg, Ni, NO_3, NO_2, pV, pH, PO_4, Na, SO_4, T-Coli, TDS, TH, Turbidity, Zn	-Five different water quality indices; WAI-WQI, CCME-WQI, UWQI, OWQI and ATI were applied to the river and the applicability of the methodologies was discussed. -ATI and CCME-WQI methods were found to be more appropriate for the 9-year water quality assessment of the river.	Bor and Elçi (2022)
WQI	Aydos River (Kastamonu)	WT, SO_4, SO_3, NO_3, BOD_5, COD, SS, NO_2, EC, salinity, Fe^{+2}, Pb^{+2}, Zn^{+2}, Cu^{+2}, Ni^{+2}, Cd^{+2}, TH, TA, PO_4^{-3}, NH_4	-The lowest WQI value of Aydos River was 55.6 in December and the highest value was 114.3 in October. It was stated that the river is in the "good water quality" class in terms of drinking water. - It was stated that high index values may be caused by fertilizers used in agricultural activities and reaching the river through rainfall.	Mutlu and Aydın Uncumusaoğlu (2022)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI	Kızılırmak and Yeşilırmak Rivers (Samsun, Middle Black Sea Region)	pH, EC, DO, COD, TN, TP, Pb, Ni, Zn, Cu, Cd	- According to the WQI results, Kızılırmak has "poor" water quality characteristics in the spring season, while Yeşilırmak has "good" water quality characteristics only in the winter season and poor and very poor water quality characteristics in all other seasons.The results show that Kızılırmak is suitable for drinking, irrigation and industrial use, while Yeşilırmak is suitable only for irrigation.	Şimşek et al. (2022)
WQI	Yağlıdere Stream (Giresun)	WT, pH, EC, DO, NO_3, NO_2, NH_3, BOD, COD, TP, TDS, Ca, Mg, Na, K, SO_4, Cl, F, HCO_3, F, Al, CN, Hg, As, Cd, Mn, Br, Al, Zn, Cu, Fe, Se, HS, fecal coliform, total coliform	-The average WQI values in the sampling areas ranged from 42.4 (excellent water) to 7137.2 (unsuitable for drinking). -It is suggested that Yağlıdere Stream is negatively affected by geogenic and anthropogenic pressures, necessary studies should be carried out to reduce and eliminate these impacts and water quality should be monitored regularly.	Hatipoğlu Temizel (2023)
CCME-WQI	Yeşilırmak River (Amasya)	pH, DO, COD, NO_3, NO_2, NH_3, NH_4, PO_4, Fe, Cu, Zn, K, SO_4, SO_3, Cl	-Calculated CWQI values, the values for the inlet waters at sampling points SW1, SW2 and SW7 in the study area were reported as 54.01, 64.46 and 56.49, respectively. It was revealed that pollutants like Tersakan negatively affected the water quality of the Yeşilırmak River, just like the Çorlu Stream.	Konare et al. (2023)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI HPI HEI HRA	Munzur Stream (Tunceli)	Mn, Hg, As, Pb, Cr, Cd	-The water quality of Munzur Stream is in good condition in terms of drinking water and irrigation water. Although the concentrations of heavy metals such as Cu, Ni, Fe and Hg were high, they were significantly below the permissible limits under the Turkish Ministry of Forestry and Water Affairs Surface Water Quality Regulations (TSWQR). - According to HQ and HI values, Munzur Stream is safe for public health for residential uses.	Kutlu and Sarıgül (2023)
WQI NPI	Meriç–Ergene River Basin	DO, pH, EC, TDS, Salinity, NO_2, NO_3, SO_4, Cl	- Water Quality Index (WQI) results in dry (end of summer) and wet (end of winter) surface water samples collected from 9 stations in total from 5 basins, namely Anadere, Çorlu, Tunca, Meriç and Ergene Rivers, showed that the most polluted components of the basin were Çorlu Stream and Ergene River. Organic pollution risk assessment indices as follows: Çorlu Stream>Ergene River>Anadere Stream>Tunca River>Meriç River. - In order to increase the quality of aquatic life in the Meriç Ergene River Basin and ensure its sustainability, it is recommended to avoid the unconscious use of pesticides and fertilizers in the region and to prevent the untreated discharge of industrial wastewater and municipal sewage.	Tokatlı and İslam (2023)

Table 2 (continued)

Index	Rivers -Streams	Parameters	Major Findings	Reference
WQI NI NPI RPI HPI HEI HRA	Çorlu Stream (Tekirdağ)	pH, EC, DO, NH_4–N, PO_4–P, TSS, SO_4, NO_2, NO_3, BOD, TDS, turbidity, COD	- According to the water quality and heavy metal index values, it was reported that the selected stations 2 and 3 along Çorlu Stream, which is located in the Thrace Region and exposed to intense industrial pressure, were severely polluted due to domestic and industrial wastewater discharges. - Given the results of the HRA, it was estimated that non-carcinogenic risks from ingestion of combined metals in the river water can be expected at station S3 for both children and adults and at station S2 for children, and that Cr and As at station S3 may cause carcinogenic health risks for residents. - It was reported that wastewater from the Organized Industrial Zones in Çorlu and Çerkezköy districts should not be discharged into the river without treatment, unconscious use of chemical fertilizers and pesticides in the region should be avoided and water quality should be monitored regularly.	Varol and Tokatlı (2023)

Table 2 (continued)

Index	Creeks	Parameters	Major Findings	Reference
CCME – WQI	Aksu Creek (Giresun)	36 water quality parameters	- When the CWQI index values applied for 3 different station points on Aksu Stream, which is an important drinking water source of Giresun Province, were compared with the measured water quality parameter values, it was seen that the index values gave reasonable results. Index values for all three stations were found in the "poor" category. - The findings showed that in these areas on the Aksu Stream, the water is not suitable for both drinking and domestic use without treatment.	Tunç Dede and Sezer (2017)
CCME-WQI	Ankara Creek Basin	pH, EC, DO, BOD, COD, TN, NH_3-N NO_3-N, NO_2-N, TKN, TP, SO_4, Fl, Cl, Na, K, Se, Cd, Ni, Al, An, Ar, Cu, Ba, Zn, Co	- CCME-WQI results for the five stations identified in the basin indicate poor, moderate and fair water quality for Stations 1, 2 and 3 on the streams connecting to the Ankara Creek, respectively, and poor water quality for Station 4 and Station 5 on the Ankara Creek. - It was reported that the results obtained were consistent with the water quality classes of the Surface Water Regulation and also recommended that this index be used by decision makers.	Aydoğdu Kayadelen (2022)

Table 2 (continued)

Index	Creeks	Parameters	Major Findings	Reference
HPI HEI	Sukesen Creek, Başpınar Creek, Yavrucak Creek, Gölcük Creek (Mogan Lake Basin-Ankara)	Hg, As, Cd, Cr, Pb, Ni, Cu, Zn	- While the HPI value was less than 15 in all months and creeks, the highest HPI value of 13.21 was calculated in February in Başpınar Creek. Since HEI values were less than 10 (0.02-0.84), low level of heavy metal pollution was determined in terms of this index. -It has been determined that heavy metal levels in creek waters due to anthropogenic activities do not cause serious pollution for the time being, but especially Başpınar Creek is more at risk than other creeks.	Pulatsü and Latifi (2023a)

Table 2 (continued)

Index	Creeks	Parameters	Major Findings	Reference
HRA	Sukesen Creek, Başpınar Creek, Yavrucak Creek, Gölcük Creek (Mogan Lake Basin-Ankara)	Hg, As, Cd, Cr, Pb, Ni, Cu, Zn	-The THI values related to Sukesen and Başpınar Creek; in all months sampled for adults and children, in Gölcük Creek, for adults in April, and for children during the sampling period, have revealed a significant non-carcinogenic health risk probability. The calculated CR values for chromium, nickel, and arsenic indicate that exposure through the digestive tract is more risky than dermal exposure, expressing the likelihood of developing cancer in both adults and children. -In the context of potential health hazards, it was reported that the implementation of administrative measures, especially concerning heavy metal contamination in Başpınar and Sukesen Creeks, was crucial not only for the protection of public health but also for the sustainability of Lake Mogan.	Pulatsü and Latifi (2023b)

* Weighted Arithmetic Index (WAI-WQI), Canadian Council of Ministers of the Environment Water Quality Index (CCME – WQI), Oregon Water Quality Index (OWQI), Universal Water Quality Index (UWQI), National Sanitation Foundation Water Quality Index (NSF – WQI), Dinius Water Quality Index (D-WQI), Organic Pollution Index (OPI), Aquatic Toxicity Index (ATI), Water Toxicity Index (ATI), Nutrient Pollution Index (NPI), River Pollution Index (RPI), Heavy Metal Pollution Index-HPI, Heavy Metal Evaluation Index-HEI, degree of contamination index (C_{deg}).

**Water Temperature- WT (°C), dissolved oxygen – DO, oxygen saturation – OS, total dissolved solids – TDS, electrical conductivity – EC, Total hardness - TH , Total alkalinity - TA, suspended solid matter - SSM), sulphite -SO_3^{-2}, sulphate - SO_4^{-2}, chloride - Cl^- , calcium - Ca^{+2}, magnesium - Mg^{+2}, sodium - Na^+, potassium - K^+, bicarbonate - HCO_3^-, ammonium - NH_4, ammonia- NH_3, nitrate – NO_3 , nitrite – NO_2 , sulphate – SO_4 , oxidation – reduction potential – ORP, phosphate – PO_4, biological oxygen demand –BOD, chemical oxygen demand – COD, nickel -Ni, chromium - Cr, arsenic - As, zinc - Zn, lead- Pb, copper – Cu, cadmium – Cd, cyanide - CN^-, mercury - Hg, iron – Fe, manganese – Mn, selenium - Se, fluoride - F, Molybdenum - Mo, Aluminum- Al, Antimony- Sb, Boron - B , Beryllium - Be, Silver - Ag, Barium - Ba, Thallium – Tl, chlorid – Cl, lithium- Li

***(HRA): Health Risk Assessment a) The total potential non-carcinogenic health risks: Hazard Index (HI). The Total HI (THI) value is calculated as the sum of individual HIs (HIingestion + HIdermal), THI > 1: here may be a potential for adverse non-carcinogenic health effects to occur, THI values < 1: non-carcinogenic health effects are not expected b) Carcinogenic health risk (CR) CR > 1.0×10^{-4} is considered unacceptable1.0×10^{-4} < CR < 1.0×10^{-6} is considered an acceptable range depending on the exposure conditions CR<1.0×10^{-6} is considered not to have significant health effects (USEPA 2004; Mohammadi et al. 2019)

Results and Discussion

With the increasing anthropogenic activities accompanying social and economic growth, factors such as climate and hydrology can lead to the accumulation of pollutants in surface waters, causing a gradual change in water quality. In this context, maintaining water quality is crucial for the sustainable management of water resources. Water quality indices, which include multiple water quality parameters, are widely used worldwide to assess the water quality of any water body, and these indices continue to be widely used with their advantages and disadvantages. Given their relative simplicity and easily relatable outputs, WQI models are widely used for water quality assessment and many different versions have been developed to date.

It has been reported that the number of studies on water quality indices in Turkey is quite low compared to other countries, and China and India are the countries with the highest number of studies on this subject (Chidiac et al. 2023). This review, which brings together the existing studies on the subject in our country, reveals that the studies have a history of less than twenty years and have gained momentum especially in the last five years.

As seen in this study, various water quality indices have been used to assess the quality of nearly fifty different surface waters such as reservoirs, lakes, rivers and streams in Turkey. In the studies conducted to determine the suitability of nine reservoirs for drinking water, WQI was found to be used the most (Boyacıoğlu 2007; Imnesisi and Aydın 2016; Baştürk 2019; Kükrer and Mutlu 2019; Tokatlı 2020; Şimşek and Mutlu 2023), while there was one study each on Heavy Metal Pollution Indexes (Leventeli and Yalçın 2019) and Health Risk Assessment (Table 2).

Heavy Metal Pollution Indexes were used in two of the studies conducted in lakes (Atıcı et al. 2021; Şener et al. 2023), which have been less studied in terms of water quality than other surface waters.

In Turkey, river ecosystems have been the most intensively used surface water resources (Table 2). As can be seen from the table, WQI indices were widely used in thirty studies on river ecosystems, and it was found that the CCME-WQI index was preferred in six of these studies. While there are six studies (Cengiz et al. 2017; Tokatlı and Ustaoğlu 2020; Tokatlı et al. 2021a; Ustaoğlu et al. 2021; Kutlu and Sarıgül 2023; Varol and Tokatlı 2023) in Turkey concerning heavy metal indices in river ecosystems, only two studies (Varol et al. 2021; Varol and Tokatlı 2023) have been identified on health risk assessment.

It is seen that CCME-WQI index was applied in a total of two studies (Tunç Dede and Sezer 2017; Aydoğdu Kayadelen 2022) in which water quality indices were applied in creek waters. The issue of heavy metal indices and health risk assessment in stream waters was discussed in only one study (Pulatsü and Latifi 2023a, b) (Table 2).

The location-index results determined as a result of the detailed resource survey (2007-2023) ranged from "good" to "poor" depending on the selected surface water type and environmental conditions, and the researches converged on the axis that the deterioration in water quality is anthropogenic activity-oriented and the importance of managerial measures to address it. In other words, water quality index studies on inland water resources of our country have revealed the necessity to put in place sound policies with necessary control measures to prevent the increasing pollution load on surface water bodies at national scale, to protect natural ecosystem areas and to reduce potential human health risks. This result is in line with the results of studies conducted in different countries (Mishra et al. 2016, Wani et al. 2016; Deep et al. 2020; Doderovic et al. 2020; Sharma 2020; Luo et al. 2021; Cong 2022) other than Turkey.

Although the selection and weighting of variables are chosen in line with the intended use of the surface water resource in question, there are also studies that investigate the possibilities of applying specific or appropriate indices for inland water resources in our country, apart from the WQIs that

are widely used. For example, Akkoyunlu and Akiner (2012) reported that, when compared with CCME-WQI, OWQI, and NSF-WQI, modified WQI scores were more appropriate in determining pollution in the rivers feeding Sapanca Lake. Bor and Elçi (2022) compared the results of five different water quality indices (the Weighted Arithmetic Index (WAI-WQI), Environmental Water Quality Index (CCME-WQI), Canadian Council of Canada (CCME-WQI), Universal Water Quality Index (UWQI), Oregon Water Quality Index (OWQI), and Aquatic Toxicity Index (ATI)) applied to the Büyük Menderes River (Aydın Söke Basin). They noted that, based on 9 years of water quality data for the river, ATI and CCME-WQI indices were more suitable for the studied river ecosystem. Çavuş and Şen (2022) reported that the WQI values they developed for Aygır Lake can be used for water quality assessment of all freshwater resources in Lake Van Basin.

In recent studies on index applications, mathematical techniques such as principal component analysis and cluster analysis have been used to better understand the selection of parameters and their weightings (Bilgin 2018; Kükrer and Mutlu 2019; Varol 2019, 2020; Aydın et al. 2021; Tokatlı 2020, 2021; Ustaoğlu et al. 2020a, b, 2021; Tokatlı and İslam 2023; Kutlu and Sarıgül 2023) while computer-based techniques such as fuzzy interface systems and artificial neural networks have been applied to reduce the uncertainty arising from the final aggregation process (Sönmez et al. 2018). In addition, GIS techniques have also been used to assess and monitor water quality indices (Şener et al. 2017; Şimşek and Mutlu 2023).

Since the application of indices to assess the water quality of a given source is a function of time and other influencing factors, it is extremely difficult to develop a universally acceptable general water quality index. Since the development of the first WQI, modifications have been made to the calculations to obtain more accurate water quality indices and the choice of variables has also changed. Kachroud et al. (2019) stated that weighting factors for specific water uses should be determined locally.

In this context, it is thought that it is important to use similar indices including common-appropriate parameters at least on the basis of river basins and to develop time-source specific water quality indices in further studies for monitoring water quality in our country. In addition, although the indices used make it possible to compare research areas, it is also important that these studies are sustained in the coming years in order to monitor changes in these areas over time and to contribute to decision-makers in developing strategic national surface water management plans.

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