INTRODUCTION

Mangroves are fulfilling several essential ecological functions that are vital to maintaining sustainability within natural resources (Nagelkerken et al. 2000). They act as a carbon sink and a type of carbon lock in the world. Hence, it is a potential plant species to store carbon in different saline conditions (Banerjee et al. 2016). Mangrove ecosystems are acting as nurseries for aquatic life while giving enormous benefits for mankind (Karunathilake 2003). Excoecaria agallocha (milky mangrove) species belongs to the family Euphorbiaceae is prominent among other mangrove species (Arulnayagam 2020). It is small to medium size, briefly deciduous, and growing up to 10 - 30 meters (Mondal et al. 2016). There are numerous advantages taken from E. agallocha, but it considers as a poisonous tree due to skin irritation, blistering and blindness that occurred when contaminate. It is called blinding tree as this latex makes blindness when contaminating (Kumarasinghe and Seneviratne 1998). Its leaf and stem sap are used to treat leprosy, epilepsy, conjunctivitis, dermatitis and so many other diseases (Bandaranayake 1998). It consists of many pharmacological potentials and many other bioactivities including antioxidant and antiviral properties (Chan et al. 2018). Further, there is a high impact of Exocqecaria agallocha on diabetes mellitus, pathogenic microbial strains, pandemic diseases, anti-oxidant, and free radical scavenging efficiency, as well as have potential in nanoparticles biosynthesis and
using as a heavy metal bioindicator (Kaliamurthi and Selvaraj 2016). Usage of herbal stimulants is a mild stone in aquaculture and *E. agallocha* is a good herbal immune stimulant for fish and shrimps (Nagarajan et al. 2019). Each part of *E. agallocha* plant contains alkaloid, saponin, flavonoid, tannins, glycoside, phenolic compounds (Rajeswari and Bhaskara-Rao 2015). *E. agallocha* sap is a botanical pesticide that can kill pests including golden snail (*Pomacea canaliculata*), caterpillars (*Spodoptera litura*), and *Crocidolomia binotalis*. The main active ingredient presents in *E. agallocha* fruits is diterpene and can control paddy pests, *Spodoptera exigua*, Cabbage head Caterpillar (*Crocidolomia binotalis*) and many other pests (Manuwee et al. 2018). It is widely used as a larviciding agent as well as a mosquito-repelling agent (Pradeepa et al. 2015), (Thangam and Kathiresan 1993). *E. agallocha* was tested for its allelopathic effect against seed germination and seedling growth of some grain crops such as green gram, black gram, groundnut, pearl millet, and finger millet (Desai and Gaikwad 2015). Similarly, *E. agallocha* root exudates have an inhibitory effect on the hyphal growth and colonization of fungi such as *Glomus mosseae* (Kumar et al. 2007).

Due to several anthropogenic and natural pressures, these mangroves are being destroyed every year globally and need immediate conservation strategy for the restoration of degraded mangrove ecosystems (Pillai and Harilal 2018). Intensive afforestation and conservation of mangrove habitats are essential to developing fast methods to raise superior seedlings. Restoration of mangrove ecosystems, mangrove re-plantation, and propagation, declare protected zones, take public support for the conservation are effective remedies to conserve mangroves including *E. agallocha* (Mathanraj and Kaleel 2015). Rather, the nutritional limitation is also an impact for successful reforestation of *E. agallocha*. Hence, applying nutrients rationally is important to promote the survival of *E. agallocha* (Chen and Ye 2014). These species are dioecious and male and female flowers are separately born. Normally male to female trees ratio is 2.2:1, and the male to female flowers ratio at the inflorescence level is 16:1 (Karamsetty and Aluri 2018). These flowering habits and seed predation is another barrier to the natural regeneration of milky mangroves (Maharan and Basak 2020). *Calliphara nobilis* insect mainly feeds on the seeds of this tree and they are often seen in large numbers during the fruiting stage. *E. agallocha* is the preferred local food plant for *Iscadia pulchra*, *Selepa celtis* (hairy caterpillar), *Achaea janatas* (castor semi-looper or croton caterpillar) and of the genus Archips, Sauris and Phyllocnistis (Karamsetty and Aluri 2018). Less seed germination further endangers this species. Therefore, a protocol for the micropropagation of *E. agallocha* by invitro propagation is a timely need (Manickam et al. 2012). In Sri Lanka, most of the fish-aggregating devices such as brush parks are constructing using branches of *E. agallocha* species (Costa and Wijeyaratne 1994). Although *E. agallocha* is one of the 21 widespread mangrove species in Sri Lanka, there is a threat of disappearing overall composition of mangrove forests (Jayathissa 2012) and poisonous nature leads to cut down and destroy *E. agallocha* habitats.

Madu River wetland is the second largest wetland in Sri Lanka and *E. agallocha* is a readily abundant true mangrove species in the wetland (Ratnayake et al. 2017). It is a hotspot of biodiversity while 303 species of flora and 248 species of vertebrates are nest in it (Amarathunga et al. 2010). Conserving this wetland including valuable mangrove species is a need for sustaining ecological, cultural, and scenic value. It needs to create a framework on ethnobotanical traditions, uses taken by small-scale fishery communities and local people in order to conserve mangrove species. There is an essential need to develop a new growing scheme of *E. agallocha* for biomass production (Hossain et al. 2015). Developing sustainable mangrove management policies is vital to conserve biodiversity (Dahdouh-Guebas et al. 2006). Socio-economic and socio-ecological studies on mangroves are very essential to understand the importance of natural vegetation. Mangroves have a very low potential to re-establish in the cleared areas which are
stressed environments (Lugo et al. 2014). It also leads to the loss of mangrove species from the gene pool and favorable means of vegetative propagation should identify for the re-establishment of mangroves (Mathiventhan and Jayasingham 2012). Thus the present study was aimed to find out the best composition of potting media and IBA concentration for the effective rooting of stem cuttings.

MATERIALS AND METHODS

The present study was conducted in a plant house at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna from December 2020 to February 2021. A Completely Randomized Design was applied to the experiment with five replicates having five single propagators each. Nine different treatments were used, where three levels of IBA concentrations (2500, 300, and 3500 ppm) were used with three potting mixtures (original on-site lagoon silt clay: sand 1:1(w/w), sand: topsoil: coir dust: compost 4:4:4:1). The treatment with IBA hormone 2500 ppm with clay: sand 1:1(w/w) potting mixture was used as the control treatment (Eganathan et al., 2000).

A well-grown female E. agallocha plant was selected from the Madu River Ramsar site. Stem cuttings were taken from a matured, healthy female plant, and 5-10 cm lengthy semi-hardwood cuttings were selected for propagation (Fig. 1). Excess leaves were removed from the cuttings to avoid touching them inside the polythene layer of the single propagator and burning the leaves. A wax layer was applied on the top end of the cutting to avoid dehydration. The cuttings were dipped in Captan fungicide solution (PENTOX CAPTAN captan 50% (w/w) WP ) for 5 minutes prior to the hormone treatment. Three IBA (Indole Butyric Acid Water Soluble IBA-K >98% Pure) concentrations 2500ppm, 3000ppm, 3500ppm were prepared by dissolving 2.5g, 3 g, and 3.5 g in 1 liter of water respectively. Semi-hardwood cuttings were dipped in respective hormone concentrations for 2 minutes. White color polythene with 300 gauge was used to prepare single propagators (16”×8”). Electrical Conductivity (EC) and pH were checked in all three potting media prior to filling propagators.

![Figure 1: (A) Stem cutting used for vegetative propagation, (B) Single propagators with stem cuttings (C) Male plant of E. agallocha, (C) Female plant of E. agallocha](image)

All single propagators were placed in a protected house (80-82°F at night and 96-98°F in the daytime). Survival percentage, root (the average number of roots, root length, root volume, root dry weight), and shoot parameters (number of shoots, chlorophyll content) were collected 45 days after the establishment by carefully removing cuttings from potting media. Data were analyzed using SAS Package version 9.1. The vigor scales were developed for analyzing qualitative characteristics for roots and shoots as follows.

Root vigor-(no callus= 0/callus=1/callus and root initiation=2/callus and few adventitious roots=3/less than ten adventitious roots= 4/more than ten adventitious roots= 5)

Shoot vigor-(no new buds= 0/less than 3 new buds=1/less than 3 new buds and new leaves=2/more than 3 buds and more leaves=3/more buds and leaves with new branches= 4/more buds, leaves, branches and fruits= 5)

RESULTS AND DISCUSSION

All three potting media were checked for their EC and pH before the establishment of the cuttings (Table 1). The highest EC was observed in potting media with lagoon silt while the lowest was from the potting media with clay: sand 1:1. pH of all potting media
was within the range of 5.4-7.5. As reported by Zhang (2019) climate, topography, parent materials, and the horizon that variably influence the pH of the soil samples.

The survival percentage of cuttings was influenced by the potting medium and IBA concentration but, not due to their synergistic effect (Fig 2). The survival percentages were significantly greater in P1 and P3 potting media and P2 which has the lowest EC showed the lowest survival percentage. Manickam et al. 2012, Meena (2000) have found the response of male and female plant cuttings of *E. agallocha* for rooting under 75, 300, 500, and 1000 ppm concentrations of IAA and IBA hormone treatments, where rooting of female cuttings was significantly higher than that of male cuttings, and IBA treated male cuttings failed to produce roots. Eganathan et al. 2000 also showed the maximum rooting was observed when *E. agallocha* cuttings were treated with IBA alone up to 2500 ppm and higher concentrations of IBA showed a better response for rooting.

The average number of roots, root length, roots volume, and average chlorophyll content were significantly affected by the interaction effect of potting media and IBA concentration. Potting media 1 consist of lagoon silt with 3500 ppm showed the highest number of roots, root length, and roots volume. The highest chlorophyll content was recorded from potting media 3 consist of sand, topsoil, coir-dust, compost 4:4:4:1 with 3500 ppm IBA (Table 2). Root dry weight was significantly affected by the IBA concentrations. But potting media was not significantly affected for root dry weights. The significantly highest root dry weight was observed in IBA 3500 ppm while the least was recorded in the 2500 ppm IBA (Fig. 3) and this observation is collaborated by previous researches. Govindan and Kathiresan (2014) have found that higher rooting occurred in 0.5 g IBA treated Avicennia

**Table 1: The variation of pH and EC of potting media used for the study.**

| Potting media | pH at 28.1°C | EC (dS/m) |
|---------------|--------------|-----------|
| P1- Lagoon silt | 6 | 6.05 |
| P2- Clay: Sand 1:1 | 5.4 | 0.05 |
| P3- Sand: Coir-dust: Topsoil : Compost 4:4:4:1 | 7.5 | 0.6 |

**Figure 2: Survival percentage of stem cuttings in different (A) potting media and (B) IBA concentrations.** (CV = 12.4%, Means with similar letters are not significantly different from each other in α=0.05) (P1- original lagoon silt, P2- clay: sand 1:1(w/w), P3- sand, topsoil, coir-dust, compost 4:4:4:1)
Table 2: Performance of stem cuttings under the treatments

| Treatments | No. of roots | Root length (cm) | Root volume (ml) | Chlorophyll content (SPAD units) | Vigor scale for root growth | Vigor scale for shoot growth |
|------------|--------------|-----------------|-----------------|----------------------------------|-----------------------------|-----------------------------|
| T1         | 11.8<sup>c</sup> | 9.2<sup>c</sup> | 2.4<sup>c</sup> | 36.0<sup>b</sup>              | 2.3                         | 2.4                         |
| T2         | 13.8<sup>b</sup> | 9.8<sup>b</sup> | 3.5<sup>b</sup> | 38.6<sup>c</sup>              | 2.5                         | 2.6                         |
| T3         | 15.9<sup>a</sup> | 12.0<sup>a</sup> | 5.8<sup>a</sup> | 46.2<sup>b</sup>              | 2.9                         | 2.9                         |
| T4         | 6.7<sup>g</sup>  | 2.7<sup>f</sup> | 0.7<sup>f</sup> | 37.7<sup>ef</sup>             | 2.4                         | 2.5                         |
| T5         | 9.3<sup>e</sup>  | 3.3<sup>f</sup> | 1.1<sup>e</sup> | 40.6<sup>d</sup>              | 2.4                         | 2.6                         |
| T6         | 10.7<sup>d</sup> | 4.1<sup>e</sup> | 1.9<sup>d</sup> | 46.1<sup>b</sup>              | 2.5                         | 2.8                         |
| T7         | 6.8<sup>e</sup>  | 3.8<sup>e</sup> | 1.9<sup>d</sup> | 36.9<sup>g</sup>              | 2.6                         | 2.6                         |
| T8         | 6.9<sup>g</sup>  | 4.9<sup>d</sup> | 2.5<sup>c</sup> | 43.7<sup>e</sup>              | 2.7                         | 2.7                         |
| T9         | 7.8<sup>f</sup>  | 4.9<sup>d</sup> | 3.4<sup>b</sup> | 55.7<sup>a</sup>              | 2.7                         | 3.0                         |
| LSD        | 1.2           | 0.9             | 0.7             | 10.1                            |                             |                             |

(T1- 2500 ppm IBA with lagoon silt, T2- 3000 ppm IBA with lagoon silt, T3- 3500 ppm IBA with lagoon silt, T4- 2500 ppm IBA with clay: sand 1:1(w/w), T5- 3000 ppm IBA with clay: sand 1:1(w/w), T6- 3500 ppm IBA with clay: sand 1:1(w/w), T7- 2500 ppm IBA with sand, topsoil, coir-dust, compost 4:4:4:1, T8- 3000 ppm IBA with sand, topsoil, coir-dust, compost 4:4:4:1, T9- 3500 ppm IBA with sand, topsoil, coir-dust, compost 4:4:4:1)(Means with same letters in each column are not significantly different from each other in α=0.05).

Officinalis and *E. agallocha* cuttings and Basak *et al.* (2000) also concluded that the highest number of adventitious roots was emerged from 2500 ppm IBA and 5000 ppm NAA treated *E. agallocha* stem cuttings. The average number of new shoots was significantly different among the potting media (P <0.0001). The highest number of new shoots was obtained from the P3 while the least by P1 which consists higher EC (Fig. 3). According to Rahman (2020), dominant mangrove species in Bangladesh are adversely affected by the increased salinity and top dying disease leads to a drastic reduction in mangrove density.

Figure 3: Variation of root dry weights in different IBA concentrations (A), (CV= 6.09%, and variation of average number of new shoots in different potting media (B), (CV= 20.9%, Means with similar letters are not significantly different from each other in α=0.05)
Propagation through stem cuttings is an easy and quick method that does not lead to genetic variation among the new propagules. Nevertheless, the root and shoot vigor of the new plantlets can be changed through the provision of plant growth promoters and an environment with optimum temperature, water, humidity, light, and appropriate potting media (Abbasi et al. 2014).

According to the vigor scale, the highest mean value for root growth was obtained by T3 and the highest mean value of shoot growth was obtained by T9 (Table 2). According to Maharana and Basak (2020), *E. agallocha* has high adaptability to new environments and resistance to varied abiotic and biotic stress due to the presence of high phenolic, flavonoid, proline contents. The preferable pH range of *E. agallocha* is 6.5-7, while being able to tolerate up to pH 8 (Pillai and Harilal 2018).

**CONCLUSIONS**

*E. agallocha* can successfully be propagated with stem cuttings using different potting media and IBA concentrations. P3 consists of sand, topsoil, coir-dust, compost 4:4:4:1 and P1 consists of lagoon silt showed the highest survival percentage while 3500 ppm IBA separately showed the highest survival. P1 consists of lagoon silt with 3500 ppm IBA showed the highest root growth including the number of roots, root length, and root volume. The highest chlorophyll content and the number of new shoots recorded from potting media with sand, topsoil, coir-dust, compost 4:4:4:1 with 3500 ppm IBA. *E. agallocha* can successfully be propagated with stem cuttings using 3500 ppm IBA with P1 or P3 potting media are the most effective method to propagate *E. agallocha*.

**AUTHOR CONTRIBUTION**

IRP conceptualized and designed the study. IRP and EMUIE performed the experiment. EMUIE analyzed and interpreted the data. All authors contributed in drafting the manuscript and IRP critically revised the manuscript.

**References**

Abbasi NA, Hafiz IA, Qureshi AA, Ali I and Mahmood SR 2014 Evaluating the success of vegetative propagation techniques in loquat cv. mardan. Pakistan Journal of Botany, 46(2): 579-584.

Arulnayagam A 2020 Floral Distribution, Diversity, and Ecology of Mangrove Forests in Mandaivu and Arali, Sri Lanka. Indonesian Journal of Social and Environmental Issues, 1(3):151–160. https://doi.org/10.47540/ijsei.v1i3.75

Amarathunga AAD, Sureshkumar N, Weerasekara KAWS, Wickramaarachchi WDN and Azmy SAM 2010 Study the effect of salinity and nutrients for the growth of *Najas marina* and its impact to aquatic
biodiversity in Madu Ganga Ramsar wetland in Sri Lanka. In Proceedings of the 15th International Forestry and Environment Symposium, 26: 27.

Bandaranayake WM 1998 Traditional and medicinal uses of mangroves. Mangroves and salt marshes, 2(3): 133-148.

Banerjee K, Amin G, Fazli P, Pramanick P and Zaman S 2016. Journal of Environmental Science, Computer Science and Engineering & Technology Excoecaria agallocha: A Potential Mangrove Species in Context to Carbon Storage in High Saline Zone. 5(2): 115–120.

Basak U, Das A and Das P2000 Rooting response in stem cuttings from five species of mangrove trees: effect of auxins and enzyme activities. Marine Biology 136: 185–189. https://doi.org/10.1007/s002270050021

Chan EWC, Oshiro N, Kezuka M, Kimura N, Baba K and Chan HT 2018 Pharmacological potentials and toxicity effects of Excoecaria agallocha. Journal of Applied Pharmaceutical, 8: 166-173.

Chen Y and Ye Y 2014 Effects of salinity and nutrient addition on mangrove excoecaria agallocha. PLoS ONE, 9 (4). https://doi.org/10.1371/journal.pone.0093337

Costa HH and Wijeyaratne MJS 1994 Utilization of mangrove species in brushpark construction and their effects on Negombo Estuary fishery (Sri Lanka). Journal of Applied Ichthyology, 10(2): 96-103.

Dahdouh-Guebas F, Collin S, Seen DL, Rönnbäck P, Depommier D, Ravishankar T and Koedam N 2006 Analysing ethnobotanical and fishery-related importance of mangroves of the East-Godavari Delta (Andhra Pradesh, India) for conservation and management purposes. Journal of Ethnobiology and Ethnomedicine, 2 (1): 1-22.

Desai N and Gaikwad DK2015 Allelopathic effects of leaf litter leachates of mangrove Excoecaria agallocha L. on rice seedlings. Allelopathy Journal, 36 (2): 293-302.

Eganathan P, Rao CS and Anand A 2000 Vegetative propagation of three mangrove tree species by cuttings and air layering. Wetlands Ecology and Management, 8(4): 281-286.

Govindan T and Kathiresan K 2014 Anatomical changes during rooting of mangroves - Avicennia officinalis and Excoecaria agallocha. European Journal of Medicinal Plants, 12(4): 1534-1542.

Hossain M, Siddique MRH, Saha S and Abdullah SR 2015 Allometric models for biomass, nutrients and carbon stock in Excoecaria agallocha of the Sundarbans, Bangladesh. Wetlands ecology and management, 23(4): 765-774.

Jayathissa LP 2012 Present Status of Mangroves in Sri Lanka. In: The National Red List 2012 of Sri Lanka; Conservation Status of the Fauna and Flora. Weerakoon, D.K. & S. Wijesundara Eds., Ministry of Environment, Colombo, Sri Lanka: 197-199.

Kaliamurthi S and Selvaraj G 2016 Insight on Excoecaria agallocha: an overview. Natural Products Chemistry & Research.4: 203. doi:10.4172/2329-6836.1000203

Karamsetty HJ and Aluri JSR 2018 Ambophily in the Dioecious Weedy Mangrove Associate, Excoecaria agallocha (Euphorbiaceae). Transylvanian Review of Systematical and Ecological Research, 20(2): 15-28.

Karunathilake KMB 2003 Status of mangroves in Sri Lanka. Journal of coastal development, 7(1): 5-9.

Kumar T, Ghose M and Brahmachary R L 2007 Effects of Root Exudates of Two Mangrove Species on in vitro Spore Germination and Hyphal Growth of Glomus mosseae. In Research Journal of Botany, 2(1): 48–53). https://doi.org/10.3923/rjb.2007.48.53

Kumarasinghe SPW and Seneviratne R 1998 Skin and eye injury due to latex of...
Excoecaria agallocha. Australasian Journal of Dermatology, 39(4): 275–276. https://doi.org/10.1111/j.1440-0960.1998.tb01492.x

Lugo AE, Medina E and McGinley K 2014 Issues and challenges of mangrove conservation in the Anthropocene. Madera y Bosques, 20 (1): 11-38.

Maharana PK and Basak UC 2020 Studies of Non-Enzymatic and Growth Changes in Two Vegetatively Propagated Mangrove Species, *Excoecaria agallocha* and *Cerbera manghas* at NaCl Stress During Hardening. 9(3): 1343–1348. https://doi.org/10.21275/SR20323123319

Manickam A, Ramachandra UP and Rajaram P 2012 A micropropagation protocol for a critically endangered mangrove *Excoecaria agallocha* L. International Journal of Conservation Science, 3(2): 119-126.

Manueke J, Tarore D, Sualang D and Mamahit JEM 2018 The Usage of Fruit Extract of Lanta (*Excoecaria agallocha* L.) for Pest Control of *Paraeucosmetus* sp. (Hemiptera: Lygaeidae) on Rice Plant (*Oryza sativa* L.). International Journal of ChemTech Research, 11(10):01-07. https://doi.org/10.20902/IJCTR.2018.111001

Mathanraj S and Kaleel MIM 2015 Threats of Mangrove Flora and the Management Actions; a Case Study in Kaluwanchikudy Area. 5th International Symposium2015 South Eastern University Sri Lanka, 307–310.

Mathiventhan T and Jayasingam T 2012 Impacts of Natural and Human disturbances on the density of tree species in selected mangrove ecosystem in Batticaloa, Sri Lanka. Journal of Science and Management. Vavuniya Campus of the University of Jaffna. ISSN, 6131 (3): 107-115.

Meena D 2000 Response of male and female cuttings of *Excoecaria agallocha* to auxins under fresh water conditions. Advances in Plant Sciences, 13(2): 461-465.

Mondal S, Ghosh D and Ramakrishna K 2016 A complete profile on blind-your-eye mangrove *Excoecaria agallocha* L (Euphorbiaceae): Ethnobotany, phytochemistry, and pharmacological aspects. Pharmacognosy reviews, 10 (20): 123.

Nagarajan BD, Ajithkumar TT, Devi G, Balasundaram C and Ramasamy H 2019 Antioxidant role and immunological function of *Excoecaria agallocha* in *Amphiprion sebae* against *Vibrio alginolyticus*. Journal of Veterinary Science and Research, 1: 50–64. https://doi.org/10.36811/jvstr.2019.110007

Nagelkerken I., Van der Velde G, Gorissen MW, Meijer GJ, Vann't Hof T and Den Hartog C 2000 Importance of mangroves, sea grass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. Estuarine, coastal and shelf science, 51(1): 31-44.

Pillai NG and Harilal CC 2018 Growth Sustaining Aspects of *Excoecaria agallocha* L. for Strategic Afforestation Protocols. International Journal of Scientific Research in Biological Sciences, 5(4): 103-108.

Pradeepa P, Subalakshmi K, Saranya A, Dinesh P, Raj VA and Ramanathan T 2015 Milky Mangrove *Excoecaria agallocha* L. Plant as a source for potential mosquito larvicides. Journal of Applied Pharmaceutical Science, 5 (3): 102-105.

Rahman MM 2020 Impact of increased salinity on the plant community of the Sundarbans Mangrove of Bangladesh. Community Ecology, 21 (3): 273-284.

Rajeswari K and Bhaskara-Rao T 2015 *Excoecaria agallocha* L. (Euphorbiaceae), An overview. Journal of Chemical and Pharmaceutical Research, 7(10): 423-439
Ratnayake AS, Dushyantha N, De Silva N, Somasiri HP, Jayasekara NN, Weththasinghe SM, Samaradivakara GVI, Vijitha AVP and Ratnayake NP 2017 Sediment and physicochemical characteristics in Madu-ganga Estuary, southwest Sri Lanka. Journal of Geological Society of Sri Lanka 18 (2): 43-52.

Thangam TS and Kathiresan K 1993 Repellency of Marine Plant Extracts against the Mosquito Aedes aegypti. International Journal of Pharmacognosy, 31(4): 321-323.

Zhang Y-Y, Wu W, Liu H 2019 Factors affecting variations of soil pH in different horizons in hilly regions. PLoS ONE 14(6): e0218563. https://doi.org/10.1371/journal.pone.0218563